RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 08/09/2017

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 1R01AI132178-01
FAIN: R01AI132178

Principal Investigator(s):
Ralph S Baric (contact), PHD
Timothy Patrick Sheahan, PHD

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

Carol J Burkhart
Grants/Contracts Specialist
CB:1350 104 Airport Drive
Chapel Hill, NC 275991350

Award e-mailed to: resadminosr@unc.edu

Period Of Performance:
Budget Period: 08/09/2017 – 07/31/2018
Project Period: 08/09/2017 – 07/31/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,455,240 (see “Award Calculation” in Section I and “Terms and Conditions” in Section III) to UNIV OF NORTH CAROLINA CHAPEL HILL in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the “Terms and Conditions” is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as “Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI132178. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.” Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator’s Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Devon R. Bumbray-Quarles
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 1R01AI132178-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$154,747
Fringe Benefits	$46,236
Personnel Costs (Subtotal)	$200,983
Equipment	$273,497
Materials & Supplies	$212,343
Travel	$6,000
Other	$16,724
Subawards/Consortium/Contractual Costs	$471,000
Publication Costs	$2,000
Tuition Remission	$1,825
Federal Direct Costs	$1,184,372
Federal F&A Costs	$270,868
Approved Budget	$1,455,240
Total Amount of Federal Funds Obligated (Federal Share)	$1,455,240
TOTAL FEDERAL AWARD AMOUNT	$1,455,240
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,455,240

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$1,455,240	$1,455,240
2	$1,166,670	$1,166,670
3	$1,166,670	$1,166,670
4	$1,166,670	$1,166,670
5	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1566001393A1
Document Number:	RAI132178A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2017

IC	CAN	2017	2018	2019	2020	2021
AI	8472315	$1,455,240	$1,166,670	$1,166,670	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414A / **Released**: (b)(6) 08/03/2017
Award Processed: 08/09/2017 12:02:06 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01AI132178-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01AI132178-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI132178. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that

reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 1R01AI132178-01

This Notice of Award (NoA) includes funds for activity with **Vanderbilt University Medical Center** in the amount of **$316,000** (**$200,000** direct costs + **$116,000**F&A costs).
This Notice of Award (NoA) includes funds for activity with **University of Texas Medical Branch** in the amount of **$155,000** (**$100,000** direct costs + **$55,000** F&A costs).
*****
Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with

documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

*****

The budget period anniversary start date for future year(s) will be **August** 1.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Kelvin D. Lyons
Email: kelvin.lyons@nih.gov **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01AI132178-01

INSTITUTION: UNIV OF NORTH CAROLINA CHAPEL HILL

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$154,747	$154,747	$154,747	$154,747	$154,747
Fringe Benefits	$46,236	$46,236	$46,236	$46,236	$46,236
Personnel Costs (Subtotal)	$200,983	$200,983	$200,983	$200,983	$200,983
Equipment	$273,497				
Materials & Supplies	$212,343	$220,895	$220,895	$220,895	$220,895
Travel	$6,000	$6,000	$6,000	$6,000	$6,000
Other	$16,724	$16,724	$16,724	$16,724	$16,724
Subawards/Consortium/Contractual Costs	$471,000	$471,000	$471,000	$471,000	$471,000
Publication Costs	$2,000	$2,000	$2,000	$2,000	$2,000
Tuition Remission	$1,825	$1,825	$1,825	$1,825	$1,825
TOTAL FEDERAL DC	$1,184,372	$919,427	$919,427	$919,427	$919,427
TOTAL FEDERAL F&A	$270,868	$247,243	$247,243	$247,243	$247,243
TOTAL COST	$1,455,240	$1,166,670	$1,166,670	$1,166,670	$1,166,670

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	55.5%	55.5%	55.5%	55.5%	55.5%
F&A Cost Base 1	$488,050	$445,482	$445,482	$445,482	$445,482
F&A Costs 1	$270,868	$247,243	$247,243	$247,243	$247,243

PI: **Baric, Ralph S**	Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV	
Received: 09/30/2016	FOA: AI16-034	Council: 05/2017
Competition ID: FORMS-D	FOA Title: PARTNERSHIPS FOR COUNTERMEASURES AGAINST SELECT PATHOGENS (R01)	
1 R01 AI132178-01	Dual:	Accession Number: 3973211
IPF: 578206	Organization: UNIV OF NORTH CAROLINA CHAPEL HILL	
Former Number:	Department: Epidemiology	
IRG/SRG: ZAI1 LR-M (M2)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 1,241,271 Year 2: 966,654 Year 3: 966,654 Year 4: 953,264 Year 5: 885,764	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Ralph Baric	University of North Carolina at Chapel Hill	PD/PI
Timothy Sheahan	University of North Carolina at Chapel Hill	MPI
(b)(6); (b)(3):7 U.S.C. § 8401	University of North Carolina at Chapel Hill	Co-Investigator
	University of North Carolina at Chapel Hill	Co-Investigator
	Vanderbilt University Medical Center	Co-Investigator
	Vanderbilt University Medical Center	Co-Investigator
	University of Texas Medical Branch	Co-Investigator

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2016-09-30

Application Identifier

5. APPLICANT INFORMATION **Organizational DUNS*:** 608195277

Legal Name*: University of North Carolina at Chapel Hill
Department:
Division:
Street1*: 104 Airport Drive, CB 1350
Street2: Suite 2200
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-1350

Person to be contacted on matters involving this application
Prefix: First Name*: Carol Middle Name: J Last Name*: Burkhart Suffix:
Position/Title: Grants/Contracts Specialist
Street1*: CB:1350 104 Airport Drive
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-1350
Phone Number*: 919-962-4098 Fax Number: 919-962-5011 Email: carol_burkhart@unc.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-566001393-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

12. PROPOSED PROJECT
Start Date* 06/01/2017
Ending Date* 05/31/2022

13. CONGRESSIONAL DISTRICTS OF APPLICANT
NC-004

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Ralph Middle Name: S Last Name*: Baric Suffix:
Position/Title: Professor
Organization Name*: University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: CB:7435 Michael Hooker Res Bldg
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-7435
Phone Number*: (919) 966-3895 Fax Number: (919) 966-2089 Email*: rbaric@email.unc.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$7,605,685.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$7,605,685.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Terry Middle Name: R Last Name*: Magnuson Suffix:
Position/Title*: Vice Chancellor for Research
Organization Name*: University of North Carolina at Chapel Hill
Department: Office of Sponsored Research
Division:
Street1*: 104 Airport Dr. Ste. 2200
Street2: CB 1350
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 27599-1350
Phone Number*: (919) 966-3411 Fax Number: (919) 962-5011 Email*: resadminosr@unc.edu

Signature of Authorized Representative*
Terry R Magnuson

Date Signed*
09/30/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter1028821813.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The University of North Carolina at Chapel Hill
Duns Number:	608195277
Street1*:	104 Airport Drive, CB 1350
Street2:	Suite 2200
City*:	Chapel Hill
County:	Orange
State*:	NC: North Carolina
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	27599-1350
Project/Performance Site Congressional District*:	NC-004

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Vanderbilt University Medical Center
DUNS Number:	079917897
Street1*:	1161 21st Avenue South
Street2:	D-7235 MCN
City*:	Nashville
County:	
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	37232-2581
Project/Performance Site Congressional District*:	TN-005

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Texas Medical Branch
DUNS Number:	800771149
Street1*:	301 University Blvd
Street2:	
City*:	Galveston
County:	
State*:	TX: Texas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	77555-1070
Project/Performance Site Congressional District*:	TX-014

Funding Opportunity Number: RFA-AI-16-034. Received Date: 2016-09-30T15:14:36.000-04:00

	File Name
Additional Location(s)	

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ● **Yes** ○ **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ● **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number 00004801

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3410-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract1028821860.pdf
8. Project Narrative*	Project_Narrative1028821861.pdf
9. Bibliography & References Cited	References_Cited1028716616.pdf
10.Facilities & Other Resources	Facilities_Resources1028821866.pdf
11.Equipment	EQUIPMENT1028523188.pdf
12. Other Attachments	Product_Development_Strategy1028716612.pdf

Funding Opportunity Number: RFA-AI-16-034. Received Date: 2016-09-30T15:14:36.000-04:00

Project Summary

Zoonotic viruses, like filoviruses and coronaviruses (CoV), represent a continuous and growing threat to global public health because they unpredictably emerge causing devastating outbreaks of pandemic disease. In the 21st century, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged from zoonotic pools of viruses, causing severe disease in humans. MERS-CoV is endemic in camels in the Middle East with continuous new infections in humans. Although SARS-CoV is not currently a threat, several “prepandemic” SARS-like CoVs have been isolated from bats that replicate efficiently in human cells and are resistant to existing therapies. With the unpredictable overlap of human and wild animal ecologies, the potential for novel CoV emergence into humans is highly probable. Currently, there are no approved antiviral therapies for any human CoV infection. Broad-spectrum CoV therapies that control known human and zoonotic CoV infections would address an immediate unmet medical need and could counter future pandemic episodes. In partnership with Gilead Sciences, we have demonstrated that the nucleoside prodrug, GS-5734, is highly efficacious in inhibiting multiple human and zoonotic CoV in vitro and SARS-CoV in vivo. The primary goal of our program is to accelerate the preclinical development of GS-5734 and promote IND licensure for the MERS-CoV indication. To thoroughly evaluate the breadth of antiviral activity and predict efficacy against future emerging CoV, we will also assess efficacy against a panel of CoV representative of family-wide genetic diversity, including prepandemic zoonotic strains poised for emergence. Focusing on the highly pathogenic MERS-CoV, our unique partnership integrates: i) metagenomics and recombinant virus synthetic genome recovery, ii) primary human lung cell models, iii) cutting edge virology and biochemistry, iv) robust murine and primate models of human disease and v) state of the art metabolic and pharmacokinetic analysis. In Aim 1, we refine the pharmacokinetics, pharmacodynamics and breadth of GS-5734 through efficacy and metabolism studies in various primary human cells with a diverse array of human and zoonotic CoV and through the evaluation of in vivo efficacy in murine and non-human primate models of MERS- and SARS-CoV. In Aim 2, we select for resistance against SARS-CoV and MERS-CoV, and determine the effect of resistance on virus replication, fitness and susceptibility to treatment. In Aim 3, we determine if the mechanism of action of GS-5734 is a result of direct effects on viral RNA replication and/or alteration of antiviral immunity via deep sequencing and single molecule RNA fluorescence in situ hybridization of vehicle or drug treated infected cells and mice. We articulate a development strategy for broad-spectrum therapeutics that could be extended to a multitude of emerging viral pathogens threatening global public health.

Project Narrative

In partnership with Gilead Sciences, we aim to accelerate the preclinical development of GS-5734 and promote IND licensure. We define the pharmacokinetics, pharmacodynamics, resistance profile, efficacy breadth and mechanism of action of GS-5734 against MERS-CoV and related emerging CoV.

FACILITIES AND RESOURCES FOR THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Baric and Sheahan Laboratories

Research Environment. The Department of Epidemiology is internationally recognized as a leader in epidemiologic research and training. The Department offers research training in most specialized areas including cancer, cardiovascular diseases, environmental and occupational health, health services/clinical epidemiology, reproductive health and infectious diseases. For the fiscal year 2010/2011, the Department was awarded in excess of $28 million in sponsored funding (research, training and public service) and ranks in the top five largest units at the University of North Carolina at Chapel Hill in the area of sponsored research awards. The department's current faculty consists of 51 regular full-time faculty and 151 adjunct faculty members. The department has 218 graduate students enrolled, including 20 in the MPH program, 5 in the MSPH program, 20 in the MSCR program and 173 in the Ph.D. program. The Department of Epidemiology is headquartered in the McGavran-Greenberg Building, but most of the laboratory space is housed in the Michael Hooker Research Center. The epidemiology administrative and office space occupies 10,928 sq. ft. and provides additional classroom space. Most of the department's research staff occupies a research annex consisting of approximately 7,000 square feet of contiguous rental space in a commercial office building that is a 10-minute walk from McGavran/Greenberg Hall.

BSL2 Facility. Dr. Baric has three laboratories of ~2400 sq. ft. equipped as BL2 space in the Michael Hooker Research Center for the molecular biology proposed in the application. Dr. Sheahan has 500sq. ft. equipped as BL2 space the Michael Hooker Research Center for the molecular biology and cell culture proposed in the application. Equipment to be shared by Drs. Baric and Sheahan include gel electrophoresis equipment, power supplies, thermal cyclers, programmable heat block, water baths, CO_2 incubators (8), several -70°C freezers, two -140°C freezers, refrigerators, DNA documentation system, DNA sequencing and computer assisted sequence analysis programs, several microfuges, two Nikon microscopes with photographic and fluorescent capabilities, several class 2 biosafety cabinets, refrigerated water baths, several new IBM and Apple computers with accompanying software, a fume hood, Nuclisens reader, hybridization oven, three fluorescent inverted scopes with computer software (Olympus IX51), and a spectrophotometer. A Roche Light Cycler 480II is available for real time measurements. The laboratory has an ELISA plate reader, an illuminometer, 200 cages for animal maintenance and breeding in Seal-Safe housing, Bio Rad low pressure chromatography system, ELISA plate washer, and spectrophotometers.

BSL 3 Facility. The Baric laboratory contains two BSL3 suites (b)(3):7 U.S.C. § square feet) with enhanced features including 1) shower in/shower out facility, 2) dual anteroom access, 3) Hepa filtered exhaust, 4) redundant exhaust fans, 4) (b)(3):7 U.S.C. § 8401 (b)(3):7 U.S.C. § and 5) Techniplast SealsafeTM Hepa filtered animal housing for mice (~300 cages). Power air-purifying respirators (PAPR) and Tyvek suits are worn at all times in the BSL3 facility. One BSL3 facility is located (b)(3):7 U.S.C. § 8401 while the other is in an (b)(3):7 U.S.C. § 8401 Each facility is equipped with sterile hoods (BSCIIA), four CO_2 incubators, gel electrophoresis equipment, thermal cyclers and power supplies, and related equipment necessary for virus cultivation and molecular genetic research. The facilities each house a -70C freezer, an inverted Nikon fluorescent microscope with an assortment of filters, magnifications and digital camera, an ELISA plate reader and illuminometer. Both facilities contain rodent-sized Seal-Safe systems for maintaining animals in a Hepa-filtered Air in/out environment, exhausted into the BSL3 Hepa-filtered exhaust system. An 8 chamber Buxco plethysmography system which allows for repetitive, noninvasive measures of the number of breaths, tidal volume, airway responsiveness, enhanced pause, respiratory gases, etc. from live control and infected mice in a contained system is available in the main BSL3 laboratory (b)(3):7 U.S.C. § 8401 (b)(3):7 U.S.C. § 8401 BSL3 is a Biorad Bio-plex MAGPIX multiplex suspension array reader which facilitates multiplex measurements of proteins/cytokines in biological samples.

Departmental and University Services. The department provides cold-room, autoclave, centralized dishwashing and a darkroom with an automated developer. The University provides a variety of core services including: sequencing and deep sequencing, genomics, genotyping, oligonucleotide synthesis, histopathology, electron, light and confocal microscopy, hybridoma, transgenic mouse, structural biology, fluorescent activated cell sorter facilities (FACS), etc. typical of any world-class research institution. As a member of the Department

of Microbiology and Immunology and UNC Cancer center, our laboratory has access to these facilities and receives discounts.

(b)(6); (b)(3):7 U.S.C. § 8401 **Laboratory**

Facilities:
The human lung tissue procurement and initial cell culture studies will be performed in the (b)(6); (b)(3):7 U.S.C. § 8401 lab in the Marsico Lung Institute/CF Research Center located in Marsico Hall on the University of North Carolina at Chapel Hill (UNC-CH) campus. Overall the Marsico Lung Institute/CF Research Center occupies ~20,000 sq. ft. on the 7th, 2nd and 1st floors of Marsico Hall and 3,000 sq. ft. in the Thurston Bowles (TB) Building. (b)(6); (b)(3):7 U.S.C. § 8401 basic research laboratory currently occupies ~590 square feet of laboratory space (Marsico Hall rooms (b)(3):7 U.S.C. § 8401 (b)(3):7 U.S.C. § 8401 The laboratories are fully equipped for general laboratory tasks, tissue culture, immunostaining, in-situ hybridization, cell transfection, protein electrophoresis including Western blotting, manipulation of DNA and RNA including gene cloning, and Southern and Northern blotting. (b)(6); (b)(3):7 U.S.C. § 8401 has approved recombinant DNA protocols and BSL2 level laboratories acceptable for adeno-, retro- and lenti-viral infection of primary cells.

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(3):7 U.S.C. § whose mission is to procure tissues for isolation of primary airway epithelial cells and support all steps towards production of well-differentiated airway epithelial cell cultures. (b)(3):7 U.S.C. § 8401

(b)(3):7 U.S.C. § 8401 These laboratories are fully equipped for tissue culture and attendant general laboratory tasks, and equipment is listed below. (b)(6); (b)(3):7 U.S.C. § 8401 has approved IRB protocols enabling procurement of human tissues.

Office:
(b)(6); (b)(3):7 U.S.C. § 8401 has a ~110 sq. ft. office and ~340 sq. ft. of carrels for up to 13 laboratory members. The Core manager has a ~100 square foot office. Shared office equipment in the Marsico Lung Institute/CF Research Center includes a copier/scanner and fax machine. The Marsico Lung Institute/CF Research Center accounting and administrative staff provide support services.

Major Equipment:
The Tissue Procurement and Cell Culture Core is equipped with 7 laminar flow biological safety cabinets, 4 dual chamber CO_2 tissue culture incubators, benchtop centrifuges and inverted and dissecting microscopes. Additionally, the Core has access to a cold room, autoclaves, 18 megOhm distilled water supply, -80 and –20 freezers, and 4 liquid nitrogen cell storage tanks. The Core also has access to an offsite liquid nitrogen cell storage facility.

Multiple real-time PCR machines are available in the Department of Cell Biology and Physiology and in the Marsico Lung Institute. All small equipment for RNA extraction (microcentrifuges, vacuum hood, etc.) and protein analyses is available in (b)(6); (b)(3):7 U.S.C. § 8401 laboratory. Both the CF Center and The Department of Cell Biology and Physiology have LICOR Odyssey Systems that provide highly sensitive linear immunoblotting capabilities for protein analysis. A Neon nucleofection system is available.

Computer:
The investigators and technicians listed on this application have access to state-of-the-art personal computers for data analysis, data acquisition, and word processing. All computers are hardwired to the University network and to the Internet. Support for the University network and computers is provided by the Office of Information Services in the School of Medicine and the Electronic Services Department. All computers have appropriate word processing, spread sheet, graphics, statistics and image analysis programs. The labs have full on line access to Vector NTI genetic database software and literature searching resources. In addition, 3 printers (two of which are color printers) and a scanner are available. Data acquisition software is available for computer interface to Ussing chambers.

Other:
The Histology Core facility, located in Marsico Hall (b)(3):7 U.S.C. § 8401 provides support to the investigators from the Marsico Lung Institute/CF Research Center in all the major steps of histological procedures, from the fixation of the tissue to the further processing needed to obtain samples suitable for histological analysis. The

laboratory is equipped to process a variety of specimen, from frozen to paraffin-embedded samples. Hematoxylin & Eosin and AB-PAS staining are routinely performed. Processing of samples for transmission and scanning electron microscopy is also available.

Project investigators have open access to the Michael Hooker Imaging Facility located in Taylor Hall, which is close to Marsico Hall. The facility provides standard and advanced digital light microscopy and image processing resources to users from the UNC-CH campus on a fee for use basis. Instrumentation and instruction are provided to enable users to acquire, process and analyze images. Multiple modes of imaging are supported including fluorescence, transmitted, interference contrast, phase contrast singly or in combination. Staff is available to assist with training, operation, maintenance and trouble shooting of the equipment. Equipment relevant for this application are three confocal microscopes including a Zeiss 880 and widefield light optical microscopes, one with fluorescence capability.

Besides the (b)(6); (b)(3):7 U.S.C. § 8401 the Marsico Lung Institute/CF Research Center operates other long standing Cores, which provide material and consultative support. In addition to the Histology Core noted above, assistance is available for creation of recombinant DNA and all associated molecular techniques, via the Molecular Core. The UNC CF Center Correction Core is a world-class facility for Ussing chamber and molecular analysis of CFTR (IP-Western). As a member of the Lineberger Comprehensive Cancer Center, Dr. (b)(6); (b)(3):7 U.S.C. § 8401 has full use of multiple Cores including a tissue culture and molecular biology supply facility, automated DNA sequencing, oligonucleotide synthesis Core as well as the High Throughput Genomics Sequencing Facility and Genomics and Bioinformatics Core.

The UNC Flow Cytometry Core Facility provides state-of-the-art flow cytometry and sorting services to the entire UNC-CH research community. The Facility provides analytic flow cytometry utilizing Cytek-modified 5-color FACScan and three 9-color Dako CyAns. Sorting is provided by a Dako MoFlo, a Dako MoFlo XDP, and an iCyt Reflection. Skilled staff provide help with instrument setup, data analysis, and consultation for experiment design.

Multiple other Cores (expression analysis, proteomics etc.) are present on the UNC Campus that can be used on an “as needed” fee for service basis as the ongoing studies may demand. The University has molecular biology, cell, tissue culture, scientific, electronic and chemical storerooms from which supplies may be purchased at discounts as a result of negotiated contracts between the University and vendors.

FACILITIES AND OTHER RECOURCES – Vanderbilt University Medical Center

Vanderbilt University Medical Center (VUMC) is a top 15 medical Center in the United States. Core facilities at Vanderbilt include Sequencing, HTS screening, cell imaging, flow cytometry and others. The Division of Infectious Diseases, Department of Pediatrics at Vanderbilt has active research programs in virology, vaccinology and emerging infections.

BSL2 virology research. (b)(6); (b)(3):7 U.S.C. § 8401 and has a research program with (b)(6); (b)(3):7 U.S.C. § 8401 of continuous support for coronavirus research. (b)(6); (b)(3):7 has 1500 square feet of BSL2 laboratory space on (b)(6); (b)(3):7 U.S.C. § 8401 has space equipped with laminar flow hoods (3) CO2 incubators (8), medium, high-speed and ultracentrifuges, refrigerators, -20°C and -80°C freezers, as well as other equipment required for molecular virology studies and recombinant genetics, including electroporator, RT-qPCR (ABI), Oddessy, thermocyclers, and nanodrop spectrophotometer. Common equipment rooms include incubator / shaker, speedvac, gel dryers. Computers and printers are available to all investigators. Dr. (b)(6); (b)(3):7 U.S.C. § 8401 room dedicated to fixed and live cell fluorescence microscopy with Zeiss and Nikon live imaging microscopes and environmental chambers.

BSL3, Select Agents and Biosafety. (b)(6); (b)(3):7 U.S.C. § 8401 BSL3 suite with a BSL2 anteroom dedicated to research on SARS-CoV and MERS-CoV. VUMC and the lab are Select Agent Registered. The lab is certified and inspected to meet all requirements for 42 CFR 73 (Select Agent Program). The facility is capable of all stages of investigation requiring BSL3 with respiratory precautions and compliance with Select Agent Regulations under 42 CFR 73. The lab contains all equipment for the safe and secure use, maintenance, and documentation of MERS-CoV and SARS-CoV. These include personal protective equipment, -80 and -20 freezers, incubators, biosafety cabinets, microscopes, electroporators, autoclaves. Dr. (b)(6); (b)(3):7 U.S.C. § 8401 interacts directly with the Institutional Biosafety Officer and committee (IBC) for all questions of safety, protocols, dual use research and gain of function studies (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401

Resources –UTMB - (b)(6); (b)(3):7 U.S.C. § 8401

Laboratory:

BSL2/BSL-3/ABSL-3 Laboratory Space

(b)(6); (b)(3):7 U.S.C. § 8401 has dedicated BSL-2 laboratory space in the (b)(6); (b)(3):7 U.S.C. § 8401 He also has access to the BSL-3 and ABSL-3 facilities in the (b)(6); (b)(3):7 U.S.C. § 8401 BSL-2 laboratory and the BSL-3/ABSL-3 facilities at UTMB contain sufficient cell culture incubators, laminar flow hoods, chemical hoods, centrifuges, etc. Clinical pathology instruments including hematology, clinical chemistry, and blood coagulation analyzers are available both in (b)(6); (b)(3):7 U.S.C. § 8401 BSL-2 laboratory and in the BSL-3/ABSL-3 facilities. In addition, quantitative PCR and multiplex machines, microscopes, and an array of other equipment for monitoring and processing samples are available.

Animal:

Animal studies will be conducted in the fully equipped Biosafety Level 3 animal facility (b)(3):7 U.S.C. § 8401 (b)(3):7 U.S.C. § 8401 ***Biosafety Level 3 animal facility*** contains (b)(3):7 U.S.C. § 8401 of animal holding, procedure and support space. There are (b)(3):7 U.S.C. § 8401

(b)(3):7 U.S.C. § 8401

Computer:

(b)(6); (b)(3):7 U.S.C. § 8401 laboratory and office have four computers for supporting the work of his group

Office:

(b)(6); (b)(3):7 U.S.C. § 8401 office is located in the (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

FACILITIES AVAILABLE TO CONDUCT RESEARCH WITH SELECT AGENTS AT UTMB

UTMB has available state-of-the-art BSL-2, BSL-3, and BSL-4 laboratories and animal facilities, and we have a documented ability to conduct work in accordance with guidelines for Biosafety in Microbiological and Biomedical Laboratories and the PHS Policy on Humane Care and Use of Laboratory Animals. The DOD, CDC and USDA have approved our laboratories and AAALAC has certified our animal facilities.

(b)(3):7 U.S.C. § 8401

BSL2/BSL-3/ABSL-3 Laboratory Space

(b)(6); (b)(3):7 has dedicated BSL-2 laboratory space in the (b)(6); (b)(3):7 U.S.C. § 8401 He also has access to the BSL-3 and ABSL-3 facilities (b)(6); (b)(3):7 U.S.C. § 8401 laboratory and the BSL-3/ABSL-3 facilities at UTMB contain sufficient cell culture incubators, laminar flow hoods, chemical hoods, centrifuges, etc. Clinical pathology instruments including hematology, clinical chemistry, and blood coagulation analyzers are available both in (b)(6); (b)(3):7 U.S.C. § 8401 BSL-2 laboratory and in the BSL-3/ABSL-3 facilities. In addition,

quantitative PCR and multiplex machines, microscopes, and an array of other equipment for monitoring and processing samples are available. Dedicated BSL-3 animal rooms contain appropriate rodent cages necessary for the proposed work. Dedicated ABSL-3 necropsy rooms contain downdraft tables and specialized equipment for performing necropsies. In addition to standard light and fluorescence microscopy, the (b)(3):7 U.S.C. § offers a range of pecialized imaging equipment, including combined confocal and multiphoton microscopy imaging systems at the BSL-2 level for molecular imaging of thick specimens and intravital microscopy. The (b)(3):7 U.S.C. § also has a full complement of gel imaging capabilities as well as a traditional X-ray film processor. In addition, the campus has *in situ* confocal microscopy and endoscopic optical coherence tomography equipment.

Our animal challenge studies with select agents are conducted in a restricted access Animal BSL-3 (ABSL-3) Facility (b)(3):7 U.S.C. § 8401 We are equipped to perform studies on SARS-CoV.

Select Agents

The Office of Environmental Health and Safety (EHS) at UTMB maintains our current laboratory's registration with CDC as part of their select agent program (b)(3):7 U.S.C. § 8401 is registered with CDC to work on SARS-CoV. The HHHS Certificate of Facility Registration for Select Agents at UTMB was renewed in September of 2002. UTMB re-registered with CDC as required by new legislation, and the laboratory of (b)(3):7 U.S.C. § 8401 is in compliance with all federal regulations related to select agents. All personnel are approved by the Department of Justice and registered to work with specific select agents with CDC. The proximity of our state-of-the art BSL-2, BSL-3 and BSL-4 laboratories, experimental animal facilities, histopathogy core, and support personnel allow close collaboration and consultation among the various scientists and support personnel involved in the project. This combination is unique among U.S. universities. All shipments of select agents are received through our Environmental Health and safety office.

BSL-2/BSL-3 Facilities

The Experimental Pathology Division of the Department of Pathology is housed in the (b)(3):7 U.S.C. § 8401 and is connected to (b)(3):7 U.S.C. § Laboratory space available to the various UTMB investigators involved in these projects includes BSL-2 laboratories, plus BSL-3 laboratories for work with hazardous bacterial/viral agents. The BSL-3 laboratories were inspected by both CDC and USDA/APHIS and were approved for work with "select agents".

EQUIPMENT-UTMB

Tseng Laboratory:

(b)(3):7 U.S.C. § 8401 BSL-2 laboratory has several -80 freezers, full-size centrifuges and micro-centrifuge, Bio-Rad C-1000 Thermal cycler, Bio rad CFX96 Real time PCR platform, microscopes, incubators and gel apparatus and other equipment for Western, Northern, and Southern blotting.

GNL Equipment:

Among the more advanced instrumentation available within the GNL are: PET/CT scanner; IVIS *in vivo* imaging system; multiphoton confocal microscopy at both BSL-2 and BSL-3; digital X-ray; robotic liquid handling capabilities and other robotics instruments for assay development, a full complement of thermo-cyclers, including RT-PCR machines; flow cytometry at BSL-4 and cell sorting at BSL-3; telemetry systems for *in vivo* monitoring of various parameters; a fully equipped experimental pathology laboratory.

BSL-2/BSL-3 Facilities:

The Experimental Pathology Division of the Department of Pathology is housed in the (b)(3):7 U.S.C. § 8401 which is connected to (b)(3):7 U.S.C. § Laboratory space available to the various UTMB investigators involved in these projects includes BSL-2 laboratories, plus BSL-3 laboratories for work with hazardous bacterial/viral agents. The BSL-3 laboratories were inspected by both CDC and USDA/APHIS and were approved for work with “select agents.” Core facilities include: arthropod containment facility, a fully equipped Electron Microscopy Laboratory, and a darkroom equipped to develop X-Ray films.

Other major equipment located in this space includes: chemical and biological safety cabinets; CO2 incubators; -20ºC; -80ºC; and liquid nitrogen freezers; a Philips 525M scanning microscope and two Philips transmission electron microscopes (DM 100 and 201); a Meridian Insight confocal microscope and digital image analyses system; inverted and standard microscopes and a fluorescent microscope with photographic capabilities; a Strategene Eagle Eye II still video system; a Packard instant imaging System; a Silicon Graphics indigo graphics work station; an automatic X-Ray film developer; a Scanalytics benchtop plus scanner densitometer; a Dynatech MRX automated plate reader; a Coulter Epics C fluorescence activated cell sorter; Coulter, scintillation and gamma counters; a Perkin Elmer automated DNA sequencer; an ABI Prism 7700 Sequence Detection System; a work station for nucleotide sequence analysis; numerous thermal cyclers (including a Beckman Biomek 2000 robotic PCR system); spectrophotometers; gel electrophoresis equipment; gel dryers; isotope facilities; two ultramicrotomes; cryotomes; ultra-, superspeed-, and low-speed-centrifuges; and glassware washing and sterilization equipment.

Product Development Strategy for Partnerships for Countermeasures Against Select Pathogens (R01)

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

A. Milestones and Timelines

Milestones

1. (b)(4)

(b)(4)

4. **Resistance analysis / MOA (1Q20)**
   Understanding pathways to resistance, mechanism of action (MOA) and the phenotypes of virus variants with reduced susceptibility to GS-5734 (resistant mutants) will provide the foundation for establishing an effective and useful clinical virology program for the monitoring of treatment emergent resistant virus in clinical specimens. Our preliminary work demonstrates that resistance can be generated to GS-5734 in a cell culture model of murine CoV (mouse hepatitis virus), with mutations that arise in highly conserved residues within the RNA dependent RNA polymerase, and that resistance can be transferred to SARS-CoV upon introduction of resistance mutations into SARS-CoV. With this proposal, we aim to generate resistance mutants with MERS- and SARS-CoV in both in vitro (i.e. cell lines, primary human airway epithelial cells, etc.) and in vivo models (rodents). Using reverse genetics, we will then reengineer resistance mutations back into parent viruses to conclusively demonstrate specific amino acid changes that reproduce the resistance phenotype. The goals are to determine if there are shared genetic pathways to resistance in genetically distinct viruses, to determine if there is a loss of fitness in vitro and in vivo through the acquisition of resistance and to determine the effect of resistance on GS-5734 treatment in mouse models of CoV pathogenesis. It is essential that we understand the fitness cost (if any) and possible alterations in pathogenesis of variants with reduced susceptibility to GS-5734 to ensure safety of patients during clinical development. A detailed characterization of the in vitro and in vivo properties of virus variants with reduced susceptibility to GS-5734 will be required for completion of this milestone. This milestone will be considered complete with the submission of the resistance analysis plan to support the Phase 2 clinical program prior to unblinding of the clinical data.

Pitfalls and solutions:

GS-5734 resistant virus variants could arise frequently in vivo or show altered pathogenic properties. The potential for these virus variants to replicate better than wild type virus is unlikely based upon our previous experience with inhibitors that target viral polymerases. These compounds tend to have high genetic barriers to resistance and variants with reduced compound susceptibility tend to replicate less efficiently than wild type virus. Consequently, these variants often show reduced pathogenesis in vivo. It is possible that traditional endpoints in the animal models (i.e. virus lung titer via plaque assay, viral genome quantitation via RT-qPCR) will not be sufficiently robust to measure statistically significant differences in the pathogenesis of wild type virus compared to resistant variants, necessitating the use of lethal dose 50 determinations, both in young and aged animals. Alternatively, increasing the sample size might provide sufficient statistical power to generate statistical significance. As part of this program, we will develop new very sensitive methods to monitor virus replication based on in vivo bioluminescent imaging, which should improve the sensitivity of the assessment of resistant variants in vivo.

(b)(4)

B. Product Development Plan

The main objective of the GS-5734 coronavirus program is to develop a therapeutic for the treatment of Middle East Respiratory Syndrome Coronavirus (MERS-CoV). The goal of this research project is to provide the necessary preclinical data to support our NDA filing. Our plan for this collaboration is to generate additional preclinical data describing the metabolism and distribution of GS-5734 and metabolites in tissues relevant to MERS-CoV infection. In addition, data will be generated describing the biological properties of drug resistant variants that will lay the foundation for our clinical virology program. Gilead plans to leverage existing preclinical, product manufacturing and clinical data generated from our Ebola virus program to support an expanded indication for treatment of MERS-CoV patients. We will seek FDA guidance after review of the current data prior to initiating our clinical program in MERS-CoV patients.

A summary of the current development status for GS-5734 for treatment of Ebola virus infection is described below. Gilead plans to reference this information to support the IND for the MERS-CoV indication.

(b)(4)

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(4)

of the Freedom of Information and Privacy Act

(b)(4)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Ralph Middle Name S Last Name*: Baric Suffix:

Position/Title*: Professor
Organization Name*: University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: CB:7435 Michael Hooker Res Bldg
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: (919) 966-3895 Fax Number: (919) 966-2089

E-Mail*: rbaric@email.unc.edu

Credential, e.g., agency login: (b)(6)

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1982

Attach Biographical Sketch*: File Name: Biosketch_Baric1028821868.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Timothy Middle Name Patrick Last Name*: Sheahan Suffix:

Position/Title*: Research Assistant Professor
Organization Name*: University of North Carolina at Chapel Hill
Department: Epidemiology
Division: School of Public Health
Street1*: 3109 Michael Hooker Res Bldg
Street2: CB# 7435
City*: Chapel Hill-7435
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: 919-843-8558 Fax Number:

E-Mail*: sheahan@email.unc.edu

Credential, e.g., agency login: (b)(6)

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2008

Attach Biographical Sketch*: File Name: Biosketch_Sheahan1028523125.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b)(6); (b)(3):7 U.S.C. § 8401 Suffix:

Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7435

Phone Number*: (b)(6); (b)(3):7 U.S.C. § 8401

E-Mail*: (b)(6); (b)(3):7 U.S.C. § 8401

Credential, e.g., agency login: (b)(6); (b)(3):7 U.S.C. § 8401

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 2001

Attach Biographical Sketch*: File Name: Biosketch (b)(3):7 U.S.C. § 8401

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: (b)(6); (b)(3):7 U.S.C. § 8401 Suffix:

Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*: Chapel Hill
County: Orange
State*: NC: North Carolina
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 27599-7248

Phone Number*: (b)(6); (b)(3):7 U.S.C. § 8401
E-Mail: (b)(6); (b)(3):7 U.S.C. § 8401

Credential, e.g., agency login: (b)(6); (b)(3):7 U.S.C. § 8401

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1985

Attach Biographical Sketch*: File Name: Biosketch_(b)(6); (b)(3):7 U.S.C. § 8401

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b)(6); (b)(3):7 U.S.C. § 8401 Suffix: MD

Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*: Nashville
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37232-2581

Phone Number*: (b)(6); (b)(3):7 U.S.C. § 8401 Fax Number:
E-Mail*: (b)(6); (b)(3):7 U.S.C. § 8401

Credential, e.g., agency login: (b)(6); (b)(3):7 U.S.C. § 8401

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: MD/PhD Degree Year: 2001

Attach Biographical Sketch*: File Name: (b)(6); (b)(3):7 U.S.C. § 8401

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b)(6); (b)(3):7 U.S.C. § 8401 Suffix: MD

Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*: Nashville
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37232-2581

Phone Number*: (b)(6); (b)(3):7 U.S.C. § 8401
E-Mail*: (b)(6); (b)(3):7 U.S.C. § 8401

Credential, e.g., agency login: (b)(6); (b)(3):7 U.S.C. § 8401

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: MD Degree Year: 1980

Attach Biographical Sketch*: File Name: Bio (b)(6); (b)(3):7 U.S.C. § 8401

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: (b)(6); (b)(3):7 U.S.C. § 8401 Suffix:

Position/Title*:
Organization Name*:
Department:
Division:
Street1*:
Street2:
City*: Galveston
County:
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 77555-1070

Phone Number*: (b)(6); (b)(3):7 U.S.C. § 8401 Fax Number:

E-Mail*: (b)(6); (b)(3):7 U.S.C. § 8401

Credential, e.g., agency login: (b)(6); (b)(3):7 U.S.C. § 8401

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: PhD Degree Year: 1997

Attach Biographical Sketch*: File Name: Biosketch_ (b)(6); (b)(3):7 U.S.C. § 8401

Attach Current & Pending Support: File Name:

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: **RALPH STEVEN BARIC**

eRA COMMONS USER NAME (credential, e.g., agency login): (b)(6)

POSITION TITLE: **PROFESSOR**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
N.C. State University, Raleigh, NC	BS	1977	Zoology
N.C. State University, Raleigh, NC	PhD	1982	Microbiology
University of Southern CA, School of Med,(Los Angeles, CA)	Post-Doc	1986	Microbiology

A. Personal Statement: The Baric laboratory uses genetic, biochemical, molecular and immunologic approaches to study the molecular mechanisms regulating viral evolution, virus immunity, virus-host interactions and vaccine mediated protective immunity using coronaviruses (CoV), noroviruses and flaviviruses (Dengue) as models. We use SARS-CoV and MERS-CoV as models to address fundamental questions in genetics, structure-function analyses, entry and cross species transmission, fidelity regulation, host susceptibility allele mapping, pathogenesis as well as therapeutic design and testing. We have used synthetic genomics and reverse genetics to create a panel of CoV molecular cDNA clones for SARS-CoV, SARS-like bat coronaviruses (SL-CoV), MERS-CoV, several human coronavirus, Dengue 1-4 and Zika virus. We have also developed key animal models of human disease, including SARS-CoV and SL-CoV pathogenesis in young and aged mice, and CRISPR gene edited mice encoding permissive mutations in the murine dipeptidyl peptidase receptor, making the animals permissive for MERS-CoV infection and disease.

The Baric laboratory has longstanding expertise in Coronavirus evolution, replication, animal model development and pathogenesis. In collaboration with the (b)(6); (b)(3):7 U.S.C. § 8401 Lab at Vanderbilt University, we have demonstrated the first RNA proof-reading complex in an RNA virus. The Baric and (b)(6); (b)(3):7 U.S.C. § 8401 laboratories have published 20 peer reviewed papers together over 20 yrs. The Baric laboratory pioneered the use of primary human airway epithelial cells to understand virus-host interaction networks associated with innate immune antagonism, innate immune control, virus receptor interactions and mechanisms of apoptotic cell killing. Dr. Baric has worked closely with Dr. (b)(6); (b)(3):7 in the past investigating virus host interaction networks elicited during SARS-CoV infection of Calu3 cells (1 paper), noting that Dr. (b)(6); (b)(3):7 is a renown coronavirologist who has developed numerous animal models of human disease, including primates. Over the past year, we have worked closely with Gilead Sciences to preclinically evaluate antivirals to treat CoV. Thus, the assembled research team provides complementary expertise that are essential for program wide success.

Qualifications by Publication: ~260 peer reviewed publications, H-Index: 71, i10 index: 182. http://www.ncbi.nlm.nih.gov/sites/myncbi/ralph.baric.1/bibliography/40583903/public/?sort=date&direction=ascending.

1. Scobey T, Yount BL, Sims AC, Donaldson EF, Agnihothram SS, Menachery VD, Graham RL, Swanstrom J, Bove PF, Kim JD, Grego S, Randell SH, **Baric R.S**. 2013. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. **PNAS USA**.110(40):16157-62. PMC3791741.
2. Menachery, VD, Yount, BL, Debbink, K, Agnihothram, S., Gralinski, LE, Plante, JA, Graham, RL, Scobey, T., Ge, S-Y, Donaldson, E.F., Randell, S.H., Lanzavecchia, A., Marasco, W.A., Shi, Z-L, **Baric, R.S.** 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. **Nature Medicine.** Nov 9. doi: 10.1038/nm.3985. [Epub ahead of print]. PMID:26552008.

3. Frieman MB, Chen J, Morrison TE, Whitmore A, Funkhouser W, Ward JM, Lamirande EW, Roberts A, Heise M, Subbarao K, **Baric RS**. 2010. SARS-CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. **PLoS Pathog**.8;6(4):e1000849. PMC2851658.
4. Graham RL, Becker MM, Eckerle LD, Bolles M, Denison MR, **Baric RS**. 2012. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. **Nat Med**. Dec 6;18(12):1820-6. doi: 10.1038/nm.2972. PMCID: PMC3518599.

B. Positions and Honors.

Employment Experience:

1986-1992 Assistant Professor, Department of Parasitology and Laboratory Practice and Department of Epidemiology, University of North Carolina (UNC), Chapel Hill, NC
1992-2001 Associate Professor, Departments of Epidemiology and Microbiology & Immunology, UNC Chapel Hill
2001- Professor, Departments of Epidemiology and Microbiology and Immunology, UNC Chapel Hill

Selected Awards/Honors:

2015 US Natl. Acad. Of Sciences/UK Royal Society Workshop: Raymond and Beverly Sackler U.S.-U.K. Scientific Forum on the Trends in Synthetic Biology and Gain of Function and Regulatory Implications, Nov 15-17, 2015, Chicheley, United Kingdom.
2015 US Natl. Acad. Of Sciences "China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety, and Global Health Security" September 28-30 in Beijing, China
2015 MERS-CoV Stakeholders Workshop, Invited panelist., NIH
2014 National Academy of Sciences: Working Group on Risks and Benefits of Gain of Function Research
2005-2015 Review Board, J. Virology
2008-2015 Senior Editor, Plos Pathogens
2008- Member-Biological Sciences Expert Group (BSEG)
2008 National Academy Sciences: Working Group: Gene Sequence Methods for Classification of Select Agents
2007-2008 Associate Editor, Plos Pathogens
2005-2009 Permanent Member, NIH VirB Study Section
2003 Finalist/Runner-up, World Technology Award
1989-1994 Established Investigator: American Heart Association
1984-1986.1 Harvey Weaver Scholar, National Multiple Sclerosis Society

C. **Contributions to Virology:** The Baric laboratory has made significant contributions to our understanding of all aspects of CoV biology, including: i) CoV genetics and reverse genetics for SARS-CoV, MHV, MERS-CoV, HCoV NL63, PEDV, TGEV, bat SARS-like CoV (SL-CoV), BtCoV HKU-5 and others, ii) demonstration of proof-reading activities in the CoV genome, iii) identification and characterization of bat SL-CoV with prepandemic potential, iii) coronavirus transcription mechanisms, iv) mechanisms of interferon antagonism and interferon stimulated gene expression control, v) virus host susceptibility allele mapping, vi) epitope mapping of human monoclonal antibodies, vii) identification of broad spectrum human monoclonal antibodies against SARS-CoV and MERS-CoV, viii) mouse models of human disease (MERS-CoV and SARS-CoV), ix) aging and emerging coronavirus vaccine efficacy, and x) live and attenuated vaccine design in young and aged animal models of human disease. We have also made major contributions to norovirus immunology and DENV reverse genetics. ***Some representative major contributions outside and within the CoV field include***:

1. Gralinski LE, Ferris MT, Aylor DL, Whitmore AC, Green R, Frieman MB, Deming D, Menachery VD, Miller DR, Buus RJ, Bell TA, Churchill GA, Threadgill DW, Katze MG, McMillan L, Valdar W, Heise MT, Pardo-Manuel de Villena F, **Baric RS**. Genome Wide Identification of SARS-CoV Susceptibility Loci Using the Collaborative Cross. **PLoS Genet**. 2015 Oct 9;11(10):e1005504. PMID:26452100.
2. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, **Baric R**. Human susceptibility and resistance to Norwalk virus infection. **Nat Med.** 2003;9(5):548-53. PMID:12692541.
3. Lindesmith LC, Donaldson EF, Lobue AD, Cannon JL, Zheng DP, Vinje J, **Baric RS**. Mechanisms of GII.4 norovirus persistence in human populations. **PLoS Med**. 2008 Feb;5(2):e31. PMC2235898.
4. Cockrell AS, Yount, BL, Scobey T, Jensen K, Douglas M, Beall A, Tang X-C, Marasco WA, Heise MT, **Baric RS**. 2016. A Mouse Model for MERS Coronavirus Induced Severe Respiratory Distress Syndrome. In press, **Nature Microbiology**.

C.1. Coronavirus Pathogenesis and Immunity. Our group has studied the role of virus-immune interactions in coronavirus pathogenesis.

1. Rasmussen AL, Okumura A, Ferris MT, Green R, Feldmann F, Kelly SM, Scott DP, Safronetz D, Haddock E, LaCasse R, Thomas MJ, Sova P, Carter VS, Weiss JM, Miller DR, Shaw GD, Korth MJ, Heise MT, **Baric RS**, de Villena FP, Feldmann H, Katze MG. Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. **Science**.2014 Nov 21;346(6212):987-91. PMC4241145.
2. Sheahan T, Morrison TE, Funkhouser W, Uematsu S, Akira S, **Baric RS**, Heise MT. MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV. **PLoS Pathog**. 2008 Dec;4(12): e1000240. PMC2587915.
3. Menachery VD, Eisfeld AJ, Schäfer A, Josset L, Sims AC, Proll S, Fan S, Li C, Neumann G, Tilton SC, Chang J, Gralinski LE, Long C, Green R, Williams CM, Weiss J, Matzke MM, Webb-Robertson BJ, Schepmoes AA, Shukla AK, Metz TO, Smith RD, Waters KM, Katze MG, Kawaoka Y, **Baric RS**. 2014. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. **MBio.** 2014 May 20;5(3):e01174-14. PMC4030454.
4. Gralinski LE, Bankhead A 3rd, Jeng S, Menachery VD, Proll S, Belisle SE, Matzke M, Webb-Robertson BJ, Luna ML, Shukla AK, Ferris MT, Bolles M, Chang J, Aicher L, Waters KM, Smith RD, Metz TO, Law GL, Katze MG, McWeeney S, **Baric RS**. Mechanisms of severe acute respiratory syndrome coronavirus-induced acute lung injury. **MBio**. 2013 Aug 6;4(4). pii: e00271-13. PMC3747576.

C.2. Coronavirus Innate Immunity/Animal Models. Our group has studied coronavirus host range expansion using experimental evolution and SARS-CoV, MERS-CoV, civet SL-CoV, bat SL-CoV, and bat CoV HKU5 as models. In many instances, this first required the synthetic reconstruction of civet and bat CoV from in silico sequence databases, recovery of recombinant bat viruses for the first time, and then characterization of the virus host range phenotypes both in vitro and in vivo. Applications of experimental evolution have typically focused on understanding the molecular mechanisms associated with virus-receptor interactions in viral persistence, virus innate immune interactions, and mechanisms govering increased virulence in mice.

1. Agnihothram S, Yount BL Jr, Donaldson EF, Huynh J, Menachery VD, Gralinski LE, Graham RL, Becker MM, Tomar S, Scobey TD, Osswald HL, Whitmore A, Gopal R, Ghosh AK, Mesecar A, Zambon M, Heise M, Denison MR, **Baric RS**. A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain **HKU5** variant. **MBio**. 2014 Mar 25;5(2):e00047-14. PMC3977350.
2. Sheahan T, Rockx B, Donaldson E, Corti D, **Baric R**. Pathways of cross-species transmission of synthetically reconstructed zoonotic severe acute respiratory syndrome coronavirus. **J Virol**. 2008 Sep;82(17):8721-32. PMC2519660
3. Becker MM*, Graham RL*, Donaldson EF, Rockx B, Sims AC, Sheahan T, Pickles RJ, Corti D, Johnston RE, **Baric R**[1], Denison MR[1]. Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. **Proc Natl Acad Sci U S A**. 2008 Dec 16;105(50):19944-9. PMC2588415.
4. Sims AC, Tilton SC, Menachery VD, Gralinski LE, Schäfer A, Matzke MM, Webb-Robertson BJ, Chang J, Luna ML, Long CE, Shukla AK, Bankhead AR 3rd, Burkett SE, Zornetzer G, **Tseng** CT, Metz TO, Pickles R, McWeeney S, Smith RD, Katze MG, Waters KM, **Baric** RS. Release of severe acute respiratory syndrome coronavirus nuclear import block enhances host transcription in human lung cells. **J Virol**. 2013 Apr;87(7):3885-902. PMC3624188.

C.3. Virus Genetic Platforms. The Baric laboratory has pioneered strategies for performing reverse genetic analyses in coronaviruses and dengue viruses. Several coronavirus infectious cDNA clones are available in the lab, including recently emerged strains like SARS-CoV, MERS-CoV, conventional human and model coronaviruses, and several bat coronaviruses with pandemic potential. The availability of these genetic platforms allows for detailed studies into the role of viral genes in pathogenesis, innate immune antiviral immunity, vaccine performance and design, virus-receptor interactions, entry and virus evolution.

1. Yount, B, Curtis, K., Fritz L, Hensley, L., Jahrling P., Prentice E., Denison M., Geisbert T and **Baric, RS**. 2003. Reverse Genetics with a full length infectious cDNA for the SARS Coronavirus. **Proc Natl Acad Sci USA** 100(22):12995-13000. PMCID: PMC240733.
2. Rockx B, Sheahan T, Donaldson E, Harkema J, Sims A, Heise M, Pickles R, Cameron M, Kelvin D, **Baric R**. Synthetic reconstruction of zoonotic and early human severe acute respiratory syndrome coronavirus isolates that produce fatal disease in aged mice. **J Virol.** 2007 Jul;81(14):7410-23. PMC1933338.
3. Huynh J, **Li** S, Yount B, Smith A, Sturges L, Olsen JC, Nagel J, Johnson JB, Agnihothram S, Gates JE, Frieman MB, **Baric** RS, Donaldson EF. Evidence supporting a zoonotic origin of human coronavirus strain NL63. **J Virol**. 2012 Dec;86(23):12816-25. PMC3497669

4. Donaldson EF, Yount B, Sims AC, Burkett S, Pickles RJ, **Baric** RS. Systematic assembly of a full-length infectious clone of human coronavirus NL63. **J Virol**. 2008 Dec;82(23):11948-57. PMC2583659.

C4. Virus Vaccine Design and Antiviral Immunotherapy. Viruses are major causes of human morbidity and mortality worldwide. We have used structure-guided immunogen design and epitope exchange to broaden immunogenicity and build multivalent immunogens for increased vaccine breadth and diagnostic potential.

1. Deming, D.J., Sheahan, T., Heise, M, Yount, B., Davis, N., Sims, A., Suthar, M, Harkema J. Whitmore, A., Pickles R, West, A., Donaldson, E., Curtis, K., Johnston, RE, and **RS. Baric**. 2006. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. **PLoS Med** 3(12)e525 PMCID: PMC1716185.
2. Tang XC, Agnihothram SS, Jiao Y, Stanhope J, Graham RL, Peterson EC, Avnir Y, Tallarico AS, Sheehan J, Zhu Q, **Baric RS**, Marasco WA. 2014. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. **PNAS USA**. 2014 May 13;111(19):E2018-26. PMC4024880
3. Lindesmith LC, Ferris MT, Mullan CW, Ferreira J, Debbink K, Swanstrom J, Richardson C, Goodwin RR, Baehner F, et al. 2015. Broad blockade antibody responses in human volunteers after immunization with a multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial. **PLoS Med.** 2015 Mar 24;12(3):e1001807 PMC4371888.
4. Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, Funkhouser W, Gralinski L, Totura A, Heise M, **Baric RS**. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. **J Virol.** 2011 Dec;85(23):12201-15. PMC3209347

D. Research Support.

1. **U19 AI100625 (Baric, Heise MPI) 08/05/2012-7/31/2017**
   NIH/NIAID : Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
   The Collaborative Cross mouse resource is designed to untangle complex genetic interactions and to identify novel polymorphic genes regulating immune responses to SARS, influenza and West Nile viruses. These studies will identify genetic interactions that shape complex immune phenotypes after infection.
2. **U19 AI107810 (PI: Baric) 07/01/13-06/30/18**
   NIH/NIAID Characterization of novel genes encoded by RNA and DNA viruses
   We test the hypothesis that RNA and DNA viruses encode novel ORFs that target common and unique host innate immune targets to manipulate virus replication efficiency and severe disease outcomes.
   U19 AI 107810-Supplement (PI: Baric) 09/01/14-05/31/15
   NIH/NIAID Characterization of novel genes encoded by RNA and DNA viruses
   One year administrative supplement to identify viral gene products encoded by pathogenic human viruses that manipulate the host protein synthesis machinery and related signaling pathways.
3. **R01 AI 107731 (PI: De Silva) 07/01/13-06/30/17**
   NIH/NIAID Molecular Basis of Dengue Virus Neutralization by Human Antibodies
   These studies proposed here are directly relevant to developing simple assays to predict the performance, safety and efficacy of the leading dengue vaccine candidates Role: Co-Investigator
4. **R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17**
   Vanderbilt Univ./NIH/NIAID Determinants of Coronavirus Fidelity in Replication and Pathogenesis
   We test the hypothesis nsp14 functions in maintaining high replication fidelity during coronavirus infection and alters in vivo pathogenesis outcomes.
5. **U19-AI106772-02 (PI: Kawaoka) 08/01/13-05/31/18**
   Univ of Wisconsin/NIH/NIAID Modeling Host Responses to Understand Severe Human Virus
   The proposed studies acquire systems biology datasets during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window. Role: Project Investigator
6. **HHSN272201000019I-HHSN27200003 (PI: Palese) 09/30/13-04/30/16**
   MSSM/NIH MERS-CoV Mouse Model for Vaccine & Therapeutic Testing (Task Order A57)
   Specific Aims: Use generation of transgenic mice and modifications to the MERS-CoV genome to identify a mouse model for MERS-CoV that recapitulates human disease phenotypes for evaluating vaccine platforms and therapeutics. Role: Consortium PI, Director Task Order A57)
7. **U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19**
   UAB/NIH/NIAID Antiviral Drug Discovery and Development Center

The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.Role: Co-PI: Project 2.

8. **U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19**
   Columbia/NIH/NIAID Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
   The goal is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection. Role: Project 1 Leader

10. **Not Assigned (PI: Baric) 01/27/2015-09/16/2015**
    PNNL/DHS Generation of Predictive Models of Viral Pathogenesis
    Using advances in transcriptomics, proteomics, and metabolomics, we will identify changes in the virus-host interaction expression networks associated with DENV infection of Aedes aegypti cells or human immune cells in vitro, the latter model after natural receptor-mediated or after ADE mediated entry.

11. **Not assigned (PI: deSilva) 02/01/2015-01/31/18**
    (b)(4)
    The dengue human infection model: Defining correlates of protection and advancing vaccine development
    The Baric laboratory uses recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after vaccination and challenge. Role: Co-Investigator

12. **R01 AI110700 (PI: Baric) 04/20/15-03/31/20**
    NIH/NIAID Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
    The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis. Co-Director with Dr. Fang Li.

13. **1P01AI106695 - 01A1 (PI: Harris, Eva) 07/1/2015-6/30/20-**
    NIH/NIAID Protective immunity following dengue virus natural infections and vaccination
    Project 2: Aravinda deSilva and Ralph S. Baric (Co-PI).
    The goal of these studies is to identify natural correlates of protective immunity following natural infection and or vaccination.

14. 1-R01-AI125198-01 PI- de Silva 05/01/16 – 04/30/21
    National Inst. of Health.
    Preclinical assays to predict dengue vaccine efficacy
    We propose to use samples from vaccine clinical trials to identify mechanisms and correlates of protective immunity.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Sheahan, Timothy Patrick

eRA COMMONS USER NAME (credential, e.g., agency login): (b)(6)

POSITION TITLE: Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of New Hampshire	B.S.	06/1999	Microbiology/Water Resources
University of North Carolina at Chapel Hill	Ph.D.	05/2008	Virology
The Rockefeller University	Postdoctoral	03/2013	Systems Virology

A. Personal Statement

I have over a decade of experience performing translational research focused on evaluating antiviral therapeutics and elucidating virus and host factor targets for antiviral development. Through my academic and industrial training, I have acquired a broad skillset necessary to lead this program and meet our milestones. I gained extensive knowledge of coronavirus (CoV) molecular biology, pathogenesis, vaccinology, and animal model development within which to evaluate therapeutics through my graduate research at UNC Chapel Hill with Dr. Ralph S. Baric. The goals of my graduate research were to gain a better understand of the molecular mechanisms guiding CoV zoonotic emergence and to evaluate the efficacy of vaccines and antibodies against epidemic CoV and zoonotic CoV. During my graduate career I published extensively on zoonotic CoV and therapeutics (15 publications) and the skills gained executing these studies continue to be of use today. Under the guidance of Dr. Charles M. Rice at The Rockefeller University, my postdoctoral research focused on the creation of single cell systems within which to better understand the molecular mechanisms guiding hepatitis C virus (HCV) chronic infection. During my tenure at Rockefeller, I was awarded an NIH F32 fellowship through which I gained the management and leadership skills required to successfully execute grant-guided milestone driven research. To carry out proposed grant aims, I developed a systems virology approach coupling primary human hepatocyte cultures and laser-capture microdissection facilitating the isolation and transcriptional profiling of HCV infected cells at a resolution approaching that of a single cell. These studies yielded a high-impact publication in Cell Host and Microbe titled “Interferon Lambda Alleles Predict Innate Antiviral Immune Responses and Hepatitis C Virus Permissiveness.” This work was also featured on Dr. Vincent Racaniello’s popular podcast “This Week in Virology.” Additionally, I was part of team that developed a single molecule RNA fluorescence in situ hybridization (smRNA FISH) technique facilitating the quantitation of HCV RNAs and cellular RNAs associated with the innate immune response in single cells. This RNA FISH technique was applied to help define the mechanism of action (MOA) of several antiviral drugs targeting HCV. After my postdoctoral fellowship, I became an investigator at the Antiviral Discovery Performance Unit at GlaxoSmithKline (GSK) based in Research Triangle Park. At GSK, I was part of several programs focused on developing and evaluating host targeting small molecules as antivirals. Through this work, I gained expertise in whole genome siRNA screens and triage of hits, antiviral assay development, and design of in vivo efficacy studies. Importantly, I became fluent in the language of preclinical drug development through interactions with experts in drug metabolism, pharmacokinetics, drug safety and toxicology. I also led a three-way public private partnership between GSK, Perkin Elmer and the University of Wisconsin at Madison to develop in vivo imaging technology that facilitated the imaging of virus replication and pulmonary inflammation in live animals. Not only did this program result in a publication on in vivo imaging techniques for influenza virus, but I also gained

expertise in managing research collaborations between academia and industry which continues to be of great value. In July of 2015, I became research faculty at UNC Chapel Hill in the Department of Epidemiology focusing on broad-spectrum therapeutic approaches targeting CoV with the long-term goal of developing vaccines, therapeutic antibodies and small molecules to prevent future pandemics. To achieve these goals, I have been playing a lead role guiding a collaborative research project between UNC, Vanderbilt and Gilead Sciences, Inc. evaluating a prodrug nucleoside analog, GS-5734, to treat CoV. The current application builds upon this work in order to accelerate the preclinical development of GS-5734 to treat MERS-CoV and zoonotic CoV that may emerge in the future.

B. Positions and Honors

Positions and Employment

1999-2001 Laboratory Technician, Harvard Gene Therapy Initiative, Harvard Medical School, Boston, MA.
2001-2003 Laboratory Technician, Tissue Engineering Laboratory of Joseph Vacanti. Massachusetts General Hospital, Boston, MA.
2003-2008 Graduate Student, Laboratory of Ralph S. Baric, University of North Carolina, Chapel Hill, NC.
2008-2014 Postdoctoral Fellow, Laboratory of Charles M. Rice, The Rockefeller University, NY, NY.
2014-2015 Investigator, Antiviral Discovery Performance Unit, GlaxoSmithKline, RTP, NC.
2015- Research Assistant Professor, Department of Epidemiology, University of North Carolina, Chapel Hill, NC.

Other Experience and Professional Memberships

2002- Member, American Society for Microbiology
2007- Member, American Society for Virology

Honors

1998 Gordon Byers Scholarship for an Outstanding Water Resources Student.
2002 Partners in Excellence Award, Massachusetts General Hospital.
2009 Ruth L. Kirschstein National Research Service Award (Postdoctoral Fellowship).
2015 Third Place Regional GSK Beautiful Biology Award. *"In vivo imaging: A new platform to accelerate drug discovery at the host/pathogen interface".*
2015 Second Place Global GSK Beautiful Biology Award. *"In vivo imaging: A new platform to accelerate drug discovery at the host/pathogen interface".*

C. Contributing to Science

1. My early work focused on the molecular mechanisms guiding zoonotic CoV emergence and viral/host determinants of SARS-CoV pathogenesis. We elucidated mutations in the SARS-CoV required to increase pathogenesis in mice and shift zoonotic CoV host range to infect human cells. The mouse models created through these studies continue to be of use today as we evaluate new therapies targeting CoV. Moreover, these studies provided the technical foundation for synthetic genome design and recovery of recombinant zoonotic CoV, which has since been repeated multiple times by the Baric Lab.
    a. Roberts A, Deming D, Paddock CD, Cheng A, Yount B, Vogel L, Herman BD, **Sheahan T**, Heise M, Genrich GL, Zaki SR, Baric R, Subbarao K. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathogens.* 2007 Jan;3(1):e5.
    b. **Sheahan T**, Rockx B, Donaldson E, Sims A, Pickles R, Corti D, Baric R. Mechanisms of Zoonotic SARS-CoV Host Range Expansion in Human Airway Epithelium. *Journal of Virology.* 2008 Mar;82(5):2274-85.
    c. **Sheahan T**, Rockx B, Donaldson E, Corti D, Baric R. Pathways of Cross Species Transmission of Synthetically Reconstructed Zoonotic SARS-CoV. *Journal of Virology.* 2008 Sep;82(17):8721-32.
    d. **Sheahan T**, Morrison T, Funkhouser W, Akira S, Heise M, Baric R. MyD88 is required for protection from lethal infection with a mouse adapted SARS-CoV. *PLoS Pathogens.* 2008 Dec;4(12):e1000240.
2. Throughout my career, I have placed a special emphasis on pursuing and performing translational research, which is summarized in select publications below. Multiple publications from my graduate career involved the assessment of therapeutic antibodies and vaccines intended to not only protect against SARS-CoV infection, but also protect against zoonotic strains. These studies demonstrated that multiple therapeutic platforms failed to provide broadly cross-reactive immunity required to protect against zoonotic

CoV infection. For antibodies and vaccines to be useful, they must provide broadly cross-reactive immunity to combat contemporary CoV and zoonotic CoV that emerge in the future. My work on HCV in primary human hepatocytes demonstrated that specific interferon lambda alleles within hepatocytes were associated with permissiveness to infection corroborating phenotypes seen clinically. For the first time, these data showed that genetic defects in hepatocytes likely guide the development of chronic HCV infection. Lastly, my work at GlaxoSmithKline assessing a novel host targeting small molecule antivirals demonstrated that pharmacological perturbation of innate immunity could provide broad-spectrum antiviral activity. Together, these studies highlight my commitment to translational research.

a. Zhu Z, Chakraborti S, He Y, Roberts A, **Sheahan T**, Xiao X, Hensley LE, Prabakaran P, Rockx B, Sidorov IA, Corti D, Vogel L, Feng Y, Kim JO, Wang LF, Baric R, Lanzavecchia A, Curtis KM, Nabel GJ, Subbarao K, Jiang S, Dimitrov DS. Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. Proceedings of the National Academy of Sciences USA. 2007 Jul 17;104(29):12123-8.
b. **Sheahan T**, Whitmore A, Rogers K, Ferris M, Rockx B, Funkhouser W, Donaldson E, Gralinski L, Collier M, Heise M, Davis N, Johnston R, Baric R. Successful Vaccination Strategies that Protect Aged Mice from Lethal Influenza and Lethal Heterologous SARS-CoV Challenge. *Journal of Virology.* 2011 Jan;85(1):217-30.
c. **Sheahan TP**, Imanaka N, Marukian S, Dorner M, Liu P, Ploss A, Rice CM. Interferon Lambda Alleles Predict Innate Antiviral Immune Responses and Hepatitis C Virus Permissiveness. *Cell Host and Microbe.* 2014 Feb 12;15(2):190-202.
d. Wood ER, Bledsoe R, Chai J, Daka P, Deng H, Ding Y, Harris-Gurley S, Kryn LH, Nartey E, Nichols J, Nolte RT, Prabhu N, Rise C, **Sheahan T**, Shotwell JB, Smith D, Tai V, Taylor JD, Tomberlin G, Wang L, Wisely B, You S, Xia B, Dickson H. The Role of Phosphodiesterase 12 (PDE12) as a Negative Regulator of the Innate Immune Response and the Discovery of Antiviral Inhibitors. *Journal of Biological Chemistry*. 2015 Jun 8.

3. New technologies facilitate advances in science by accelerating the pace of discovery and increasing observational resolution. The work below highlights how new technologies can refine and accelerate translational research. With in vivo bioluminescent imaging, virus replication can be visualized in live animals. When applied to drug efficacy studies, inhibition of virus replication can be observed instantaneously in a single animal over time thus eliminating the need for large cohorts and sacrifice of mice over time to measure virus replication. Antivirals often inhibit virus replication. Single molecule RNA fluorescence in situ hybridization (smRNA FISH) techniques facilitate the simultaneous quantitation of viral RNAs and cellular RNAs at the single cell level. When applied to drug development as in the publication below, this technique can be applied to define the stage of replication inhibited by an antiviral and help refine the mechanism of action.
    a. Tran V, Poole DS, Jeffery JJ, **Sheahan TP**, Creech D, Yevtodiyenko A, Peat AJ, Francis KP, You S, Mehle A. Multi-Modal Imaging with a Toolbox of Influenza A Reporter Viruses. Viruses. 2015 Oct 13;7(10):5319-27.
    b. Ramanan V, Trehan K, Ong ML, Luna JM, Hoffmann HH, Espiritu C, **Sheahan TP**, Chandrasekar H, Schwartz RE, Christine KS, Rice CM, van Oudenaarden A, Bhatia SN. Viral genome imaging of hepatitis C virus to probe heterogeneous viral infection and responses to antiviral therapies. Virology. 2016 Apr 26;494:236-247. doi: 10.1016/j.virol.2016.04.020. PMID: 27128351

Complete List of Published Work in NCBI MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40066608/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

ACTIVE:

U19AI107810 (PI: Baric) 06/21/13-05/31/18 NIH/NIAID
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 UAB/NIH/NIAID
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 Columbia/NIH/NIAID
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 (PI: Baric) 04/01/15-03/31/20 NIH/NIAID
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Completed Research Support
F32 AI 084448 Sheahan (PI) 9/1/2009 – 9/31/2012
Hepatitis C virus host interactions in micropatterned hepatocyte co-cultures. The goal of this study was to develop technology facilitating the transcriptional profiling of HCV infected primary human hepatocytes.
Role: PI

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Withheld pursuant to exemption

(b)(6) ; (b)(3):7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

Page 0054 of 1425

Withheld pursuant to exemption

(b)(6) ; (b)(3):7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(6) ; (b)(3):7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph	S	Baric		PD/PI	(b)(4); (b)(6)				9,255.00	2,401.00	11,656.00
2.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					20,481.00	5,819.00	26,300.00
3.		Timothy	Patrick	Sheahan		PD/PI					17,057.00	5,035.00	22,092.00
4.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					1,586.00	420.00	2,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **62,054.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,095.00	2,650.00	17,745.00
1	Graduate Students				7,875.00	1,558.00	9,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Scientist				13,681.00	4,263.00	17,944.00
3	Research Specialist				27,120.00	8,357.00	35,477.00
2	Research Techs				40,277.00	14,876.00	55,153.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**135,752.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**197,806.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
1 . SpectraMax M3	35,646.00
2 . Magna Lysers	11,500.00
3 . Dual Stack Incubator	9,989.00
4 . Biosafety Cabinet	9,620.00
5 . -80C Freezer	13,942.00
6 . Perkin Elmer Lumina Series III	177,300.00
7 . Abaxis Hematology Analyzer	15,500.00
Total funds requested for all equipment listed in the attached file	
Total Equipment	**273,497.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	275,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	734,131.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition	1,450.00
9 . Maintenance Contracts	5,000.00
10 . Animal Housing/Histology	11,724.00
Total Other Direct Costs	**1,029,305.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,506,608.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	52	547,530.00	284,716.00
		Total Indirect Costs	**284,716.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,791,324.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_justification1028716460.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph	S	Baric		PD/PI	(b)(4); (b)(6)				9,255.00	2,401.00	11,656.00
2.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					20,481.00	5,819.00	26,300.00
3.		Timothy	Patrick	Sheahan		PD/PI					17,057.00	5,035.00	22,092.00
4.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					1,586.00	420.00	2,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **62,054.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,095.00	2,650.00	17,745.00
1	Graduate Students				7,875.00	1,558.00	9,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Scientist				13,681.00	4,263.00	17,944.00
3	Research Specialist				27,120.00	8,357.00	35,477.00
2	Research Techs				40,277.00	14,876.00	55,153.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**135,752.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**197,806.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	275,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	734,131.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition	1,450.00
9 . Maintenance Contracts	5,000.00
10 . Animal Costs	10,604.00
Total Other Direct Costs	**1,028,185.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,231,991.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	52	496,410.00	258,133.00
		Total Indirect Costs	**258,133.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,490,124.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_justification1028716460.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph	S	Baric		PD/PI	(b)(4); (b)(6)				9,255.00	2,401.00	11,656.00
2.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					20,481.00	5,819.00	26,300.00
3.		Timothy	Patrick	Sheahan		PD/PI					17,057.00	5,035.00	22,092.00
4.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					1,586.00	420.00	2,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **62,054.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,095.00	2,650.00	17,745.00
1	Graduate Students				7,875.00	1,558.00	9,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Scientist				13,681.00	4,263.00	17,944.00
3	Research Specialist				27,120.00	8,357.00	35,477.00
2	Research Techs				40,277.00	14,876.00	55,153.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**135,752.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**197,806.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	275,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	734,131.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition	1,450.00
9 . Maintenance Contracts	5,000.00
10 . Animal Costs	10,604.00
Total Other Direct Costs	**1,028,185.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,231,991.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	52	496,410.00	258,133.00
		Total Indirect Costs	**258,133.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,490,124.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_justification1028716460.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph	S	Baric		PD/PI	(b)(4); (b)(6)				9,255.00	2,401.00	11,656.00
2.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					20,481.00	5,819.00	26,300.00
3.		Timothy	Patrick	Sheahan		PD/PI					17,057.00	5,035.00	22,092.00
4.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					1,586.00	420.00	2,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **62,054.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,095.00	2,650.00	17,745.00
1	Graduate Students				7,875.00	1,558.00	9,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Scientist				13,681.00	4,263.00	17,944.00
3	Research Specialist				27,120.00	8,357.00	35,477.00
2	Research Techs				40,277.00	14,876.00	55,153.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**135,752.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**197,806.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 608195277
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	275,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	713,376.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition	1,450.00
9 . Maintenance Contracts	5,000.00
10 . Animal Costs	10,604.00
Total Other Direct Costs	**1,007,430.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,211,236.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	52	496,410.00	258,133.00
		Total Indirect Costs	**258,133.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,469,369.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_justification1028716460.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph	S	Baric		PD/PI	(b)(4); (b)(6)				9,255.00	2,401.00	11,656.00
2.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					20,481.00	5,819.00	26,300.00
3.		Timothy	Patrick	Sheahan		PD/PI					17,057.00	5,035.00	22,092.00
4.		(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator					1,586.00	420.00	2,006.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **62,054.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			15,095.00	2,650.00	17,745.00
1	Graduate Students				7,875.00	1,558.00	9,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Staff Scientist				13,681.00	4,263.00	17,944.00
3	Research Specialist				27,120.00	8,357.00	35,477.00
2	Research Techs				40,277.00	14,876.00	55,153.00
8	**Total Number Other Personnel**					**Total Other Personnel**	**135,752.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**197,806.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 608195277

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	3,000.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees **Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 608195277
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: University of North Carolina at Chapel Hill

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	275,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	608,751.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Tuition	1,450.00
9 . Maintenance Contracts	5,000.00
10 . Animal Costs	10,604.00
Total Other Direct Costs	**902,805.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,106,611.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Organized Research_On Campus	52	496,410.00	258,133.00
		Total Indirect Costs	**258,133.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Darryl Mayes, 202-401-2808

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**1,364,744.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: Budget_justification1028716460.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

UNC Budget Justification

Important Note: Our budget exceeds the $750,000 year direct costs cap, recommended in the RFA. We discussed this extensively with Dr. Schaefer prior to submission, as our project involves extensive BSL3 and Select Agent Research (MERS-CoV, SARS-CoV and related emerging bat Coronaviruses). Moreover, the program includes a large number of experiments in small and large animal models of human disease. In particular, an additional ~$200,000 year is requested for primate studies at UTMB each year that fill in critical preclinical testing gaps that are necessary to inform phase 1 trains in human populations. Thus, after consultation and discussion, we were permitted to exceed this cap.

PERSONNEL

Ralph Baric, Ph.D., Co-Principal Investigator (b)(4) months). Dr. Baric will lead and supervise the in vitro drug testing for this project and in collaboration with Dr. Sheahan manage the overall direction of this highly interactive proposal. He will interact closely with Gilead, Drs. Sheahan, (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. Drs. Baric and Sheahan will share the responsibility for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with the (b)(6); (b)(3):7 U.S.C. § 8401 and (b)(6); (b)(3):7 laboratories, writing progress reports and managing fiscal matters associated with the proposal. Given the extensive interaction and collaboration with Dr (b)(6); (b)(3):7 U.S.C. § 8401 in the past, he will also lead efforts to coordinate and promote research efforts with the groups. Dr. Baric will communicate his findings with Gilead, Dr. Sheahan, (b)(6); (b)(3):7 U.S.C. § 8401 on a regular basis via both conference calls and meetings between all laboratories working on this proposal.

Timothy Sheahan, Ph.D. Co-Principal Investigator (b)(4) months). Dr. Sheahan will lead and oversee the in vivo rodent model drug testing of the project and in collaboration with Dr. Baric manage the overall direction of this highly interactive proposal. He has extensive experience working at BSL3 containment and drug testing in rodent animal models. Dr. Sheahan will oversee (b)(6); (b)(3):7 U.S.C. § 8401 to ensure daily progress on all in vivo rodent drug testing as well as assist with problem solving and experimental design. Drs. Sheahan and Baric will share the responsibility for overseeing all research staff, holding regular laboratory meetings, communicating research findings with Gilead, the (b)(6); (b)(3):7 U.S.C. § 8401, and (b)(6); (b)(3):7 U.S.C. § 8401 laboratories, writing progress reports and managing fiscal matters associated with the proposal. Dr. Sheahan will interact closely with and meet in regular scheduled conference calls/face to face meetings with Gilead, Drs. Baric, (b)(6); (b)(3):7 U.S.C. § 8401 to communicate all data and results in real time.

(b)(6); (b)(3):7 U.S.C. § 8401 **Ph.D. Co-Investigator** (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 has more than (b)(6); (b)(3):7 U.S.C. § 8401 experience working at BSL3 with primary human airway cells (b)(6); (b)(3):7 U.S.C. § will work closely with Drs. Baric and (b)(6); (b)(3):7 U.S.C. § 8401 to design and execute all testing of SARS-CoV, MERS-CoV, and various mutant strains of each virus in primary culture models of the human lung. (b)(6); (b)(3):7 U.S.C. § 8401 to ensure that the appropriate numbers of primary human lung cells and lung cell donors are available as needed for this project. (b)(6); (b)(3):7 U.S.C. § 8401 will also oversee all select agent research in the BSL3 facility (b)(6); (b)(3):7 will report findings regularly to Drs. Baric and Sheahan as well as interfacing with Gilead to discuss all drug studies.

(b)(6); (b)(3):7 U.S.C. § 8401 **Ph.D. Co-Investigator** (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 will interact with Drs. Baric, Sheahan and (b)(6); (b)(3):7 U.S.C. § 8401 to ensure that specific project needs regarding primary cell cultures are met, establish standard operating procedures for production of culture substrates and media, and create new protocols as needed. (b)(6); (b)(3):7 U.S.C. § 8401 is expert in airway biology, and has extensive datasets evaluating genomic and metagenomic changes in these cultures following various perturbations (b)(6); (b)(3):7 will supervise (b)(6); (b)(3):7 U.S.C. § 8401 in the isolation, culture and distribution of primary cells (human airway epithelium nasal epithelium, type II pneumocytes, and alveolar macrophages) for this proposal, and oversee quality control and troubleshooting. (b)(6); (b)(3):7 U.S.C. § 8401 will consult with Drs. Baric and (b)(6); (b)(3):7 on experimental design, methods, data analysis and publication, and ensure that all regulatory and reporting requirements are fulfilled for work with the primary cell isolates.

(b)(6); (b)(3):7 U.S.C. § 8401 **Ph.D.** (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 has extensive BSL3 experience in the Baric laboratory and is an expert at working with BSL3 pathogens in mice. (b)(6); (b)(3):7 will work with

Dr. Sheahan to design and execute the in vivo animal drug testing proposed in this project. (b)(6); (b)(3):7 will also work with (b)(6); (b)(3):7 U.S.C. § 8401 to ensure there is steady progress on sample processing for viral titrations and histology.

(b)(6); (b)(3):7 U.S.C. § 8401 **Ph.D.** (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 has completed BSL3 training and is now working independently in the Baric containment laboratories. (b)(6); (b)(3):7 will work with (b)(6); (b)(3):7 U.S.C. § 8401 to perform in vitro drug testing in primary cells. He will assist with the isolation and characterization of SARS-CoV and MERS-CoV strains containing resistance mutations as well as testing these mutants in the presence of drugs.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal husbandry. (b)(6); (b)(3):7 will also support Drs. Sheahan, (b)(6); (b)(3):7 U.S.C. § 8401 research efforts as needed.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 facilitates the day-to-day operations of the UNC Cystic Fibrosis Center Tissue Procurement and Cell Culture Core. In support of this proposa (b)(6); (b)(3):7 will oversee and maintain quality control of cell isolation and culture procedures from human lung specimens and assist Drs. Baric, (b)(6); (b)(3):7 U.S.C. § 8401 with the design and performance of the primary cell culture experiments.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months). (b)(6); (b)(3):7 U.S.C. § 8401 has extensive BSL3 experience and will assist with the generation of viral stocks, viral titration assays, daily BSL3 laboratory maintenance and BSL3 animal husbandry. (b)(6); (b)(3):7 will also assist with weighing and performing whole body plethysmography measurements for infected mice. (b)(6); (b)(3):7 U.S.C. § 8401 will also maintain our RAG-/- breeding colony.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 will be responsible for preparing tissue culture cells for viral titration and will work closely with (b)(6); (b)(3):7 U.S.C. § 8401 o anticipate the needs of the project (b)(6); (b)(3):7 U.S.C. § 8401 will also be responsible for purchasing supplies and supporting Drs. Sheahan, (b)(6); (b)(3):7 U.S.C. § 8401 research efforts as needed.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 will perform cell isolation human lungs dedicated to this project per year, following specified procedures. (b)(6); (b)(3):7 will maintain inventories of frozen cells, prepare reagents and custom media, order supplies and maintain laboratory records. (b)(6); (b)(3):7 is fully trained and highly experienced in the culture methods and will work closely with Drs. Baric, (b)(6); (b)(3):7 U.S.C. § 8401 to provide the specific number of cultured human airway cells designated in the projects.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months) (b)(6); (b)(3):7 U.S.C. § 8401 graduate student in the Baric/Sheahan laboratories and (b)(6); (b)(3):7 will work closely with Drs. Sheahan and (b)(6); (b)(3):7 U.S.C. § 8401 o perform drug testing with the resistance mutants in vivo once (b)(6); (b)(3):7 U.S.C had completed his BSL3 training.

Fringe Benefits: Faculty/Staff: 22.883% Social Security and Retirement; $5,659/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,310/FTE Health Insurance. Health Insurance for Graduate Research Assistants is $3,399. All fringe rates are prorated for effort.

Dr. Baric's compensation is above the NIH salary cap, the balance of his salary will be covered by departmental funds

EQUIPMENT

Spectra Max M3 ($35,646) Funds are requested to purchase a SpectraMax M3 plate reader/luminometer for our BSL3 laboratory for the SARS-CoV and MERS-CoV nano-luciferase assays. We are currently restricted to preforming these assays in one of our laboratories and this purchase will give us additional flexibility in performing the proposed in vitro experiments in this proposal.

Magna Lysers ($11,500) Processing mouse lung tissue for viral titration assays in the BL3 requires homogenization. The Roche Magna Lyser is the best homogenizer on the market for performing homogenization in a containment laboratory. However, constantly moving the equipment into and out of the

biosafety cabinet and daily decontamination causes key parts inside the machine to break frequently. We are requesting two of Magna Lysers to replace ones that will age and break over the course of the project.

Dual Stack CO2 Incubators ($9,989) Funds are requested to replace the dual stack incubator in one of our two BSL3 laboratories. The current incubators are more than ten years old and have issues with contamination that will be solved by the copper lined units we are requesting. Incubators are required for all in vitro virus studies and viral titration assays proposed in this grant.

Biosafety cabinet ($9,620) All work in the BSL3 laboratories must occur in biological safety cabinets. Funds are requested to add an additional biological safety cabinet to our existing facilities to allow enough space to perform the proposed experiments.

-80C Freezer ($13,942) Funds are requested to store the large number of viral primary cell, mouse and non-human primate samples to be generated over the course of this project.

Perkin Elmer Lumina Series III ($177,300) The IVIS Lumina III is an in vivo imaging instrument capable of measuring bioluminescence and fluorescence in live animals. Viruses can be engineered to express luciferase whose expression can be detected by the IVIS upon injection of luciferase substrate. Not only is this technology exquisitely sensitive, but it also allows for repeated measures in live animals eliminating the need to sacrifice multiple cohorts of mice over time and the traditional evaluation of virus replication in harvested tissues. Virus replication data as measured by IVIS is also obtained instantaneously in real time eliminating the wait time associated with traditional virus titration techniques. Thus, in vivo drug efficacy testing can be done faster with far fewer animals and greater sensitivity thus fulfilling the principles of the 3Rs (reduction, refinement, replacement) that guide humane animal research. This technology will revolutionize in vivo efficacy testing.

Abaxis Hematology Analyzer ($15,500) The Vetscan HM5c is a 5-part differential hematology analyzer displaying a comprehensive 22-parameter complete blood count (CBC). Since similar blood panels are collected in routine human clinical practice, the data obtained from the HM5c is inherently translatable. Accurate measurement of CBC should prove to be a valuable biomarker of antiviral treatment success or failure since blood cell populations in humans and mice infected with SARS and MERS-CoV are modulated during infection.

SUPPLIES

Cell culture, Serum, and media ($50,000/year) Funds are requested for media, serum and related cell culture supplies to maintain Vero cells (titering) in culture to measure virus growth kinetics and to characterize mutant strains containing potential resistance mutations.

BSL3 protective gear ($30,000/year) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Plasticware ($30,000/year) Funds are requested to purchase tissue culture flasks, dishes, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing and titering virus growth in vitro.

Enzymes, kits and reagents ($40,000/year) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic

acid markers. As sequence confirmation is critical prior to assembly of full-length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of resistance mutations.

Miscellaneous ($20,000/year) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings.

Computer supplies ($10,000/year) Funds are requested for project specific computer and software upgrades over the course of the proposal.

RNA Seq ($45,000/year) RNASeq will be used to identify viral mutations that arise following passage of virus in the presence of GS-5734. Funds are requested for supplies to generate amplicon library and to prepare the library for sequencing as well as for informatics support. As such, we anticipate significant sequencing costs over the duration of this proposal.

Primary Cells ($20,000/year) Funds are requested to acquire up to 8 different primary human cell types (i.e. lung-HAE, FB, MVE, AT2; immune cell-PBMC, etc.) and testing seven different drug concentrations in triplicate. We estimate a total number of 120 wells of primary cells per year at $130 a well.

BioRad Bioplex kits ($30,000/year) Funds are requested to purchase BioRad Bioplex kits to analyze primary cell and mouse lung cytokine profiles. This data will contribute to understanding how the immune response contributes to the mechanism of action of GS-5734.

OTHER EXPENSES

Publishing ($2,000/year) Funds are requested to cover the publication of manuscripts.

Maintenance Contracts ($5,000/year) The Baric/Sheahan laboratory covers costs for maintenance on the Dracor Water Purifiers and Steris Autoclaves used in the BSL3 laboratories. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. The funds requested each year will cover a portion of these two sets of maintenance contracts.

Histology ($10,000/year) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover tissue/slide preparation and staining costs.

Animal Costs ($1,120 Year 1 only) Funds are requested to purchase 8 $RAG^{-/-}$ mice for breeding to generate the $Ces^{-/-}$ mice in Aim 3. All other mice will be sent to us from Jackson Laboratories courtesy of Gilead.

Animal Per Diem ($604/year) We anticipate breeding/acquiring from Jackson laboratory 534 mice for Aim 1, 768 mice for Aim 2, and 390 mice for Aim 3 for a total of 1,692 mice for five years. We estimate using approximately 338 mice a year. These mice will be housed in sets of 4 and will be maintained in UNC DLAM facilities for approximately 10 days prior to being moved to the BL3. 10 days x $0.71 per cage per day x 85 cages (to house 338 mice) =$604 a year.

Tuition ($1,450/year) In accordance with University policy, funds are requested to cover tuition costs for (b)(6); (b)(3):7 (b)(6); (b)(3):7 U.S.C. § 8401 graduate studies and are prorated to (b)(6); (b)(3):7 effort.

Travel

Funds are requested for the PIs and co-investigators to attend annual meetings at Gilead Sciences and one to two conferences each year. ($3,000 international and $3,000 domestic per year)

INDIRECT COST

In a DHHS agreement dated May 16, 2012, the UNC F&A rate is 52% of MTDC.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		310,270.00
Section B, Other Personnel		678,760.00
Total Number Other Personnel	40	
Total Salary, Wages and Fringe Benefits (A+B)		989,030.00
Section C, Equipment		273,497.00
Section D, Travel		30,000.00
1. Domestic	15,000.00	
2. Foreign	15,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		4,995,910.00
1. Materials and Supplies	1,375,000.00	
2. Publication Costs	10,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	3,524,520.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	7,250.00	
9. Other 2	25,000.00	
10. Other 3	54,140.00	
Section G, Direct Costs (A thru F)		6,288,437.00
Section H, Indirect Costs		1,317,248.00
Section I, Total Direct and Indirect Costs (G + H)		7,605,685.00
Section J, Fee		

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401			MD	PD/PI	(b)(4); (b)(6)				55,530.00	6,608.00	62,138.00
2 .	Dr.				MD	Co-Investigator					63,600.00	13,865.00	77,465.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **139,603.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical	(b)(4)					
1	Sr. Research Specialist				12,783.00	3,273.00	16,056.00
1	Research Assistant				15,158.00	3,880.00	19,038.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**35,094.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**174,697.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	65,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Repairs and Maintenance	5,303.00
Total Other Direct Costs	**72,303.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	58	250,000.00	145,000.00
		Total Indirect Costs	**145,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Health and Human Services, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**395,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: VUMC_BudgetJus(b)(6); (b)(3):7 U.S.C. § 8401
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401			MD	PD/PI	(b)(4); (b)(6)				55,530.00	6,608.00	62,138.00
2 .	Dr.				MD	Co-Investigator					63,600.00	13,865.00	77,465.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **139,603.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Research Specialist	(b)(4)			12,783.00	3,273.00	16,056.00
1	Research Assistant				15,158.00	3,880.00	19,038.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**35,094.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**174,697.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	65,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Repairs and Maintenance	5,303.00
Total Other Direct Costs	**72,303.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	58	250,000.00	145,000.00
		Total Indirect Costs	**145,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Health and Human Services, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**395,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: VUMC_BudgetJust (b)(6); (b)(3):7 U.S.C. § 8401
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401			MD	PD/PI	(b)(4); (b)(6)				55,530.00	6,608.00	62,138.00
2 .	Dr.				MD	Co-Investigator					63,600.00	13,865.00	77,465.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **139,603.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Research Specialist	(b)(4)			12,783.00	3,273.00	16,056.00
1	Research Assistant				15,158.00	3,880.00	19,038.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**35,094.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**174,697.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	65,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Repairs and Maintenance	5,303.00
Total Other Direct Costs	**72,303.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	58	250,000.00	145,000.00
		Total Indirect Costs	**145,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Health and Human Services, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**395,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: VUMC_BudgetJus(b)(6); (b)(3):7 U.S.C. § 8401
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401			MD	PD/PI	(b)(4); (b)(6)				55,530.00	6,608.00	62,138.00
2 .	Dr.				MD	Co-Investigator					63,600.00	13,865.00	77,465.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **139,603.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Research Specialist	(b)(4)			12,783.00	3,273.00	16,056.00
1	Research Assistant				15,158.00	3,880.00	19,038.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**35,094.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**174,697.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	65,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Repairs and Maintenance	5,303.00
Total Other Direct Costs	**72,303.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	58	250,000.00	145,000.00
		Total Indirect Costs	**145,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Health and Human Services, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**395,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: VUMC_BudgetJust(b)(6); (b)(3):7 U.S.C. § 8401
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 0799178970000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401			MD	PD/PI	(b)(4); (b)(6)				55,530.00	6,608.00	62,138.00
2 .	Dr.				MD	Co-Investigator					63,600.00	13,865.00	77,465.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **139,603.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Research Specialist	(b)(4)			12,783.00	3,273.00	16,056.00
1	Research Assistant				15,158.00	3,880.00	19,038.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**35,094.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**174,697.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 0799178970000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	65,000.00
2. Publication Costs	2,000.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Repairs and Maintenance	5,303.00
Total Other Direct Costs	**72,303.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**250,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Costs	58	250,000.00	145,000.00
		Total Indirect Costs	**145,000.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Health and Human Services, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**395,000.00**

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: VUMC_BudgetJust(b)(6); (b)(3):7 U.S.C. § 8401
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

DETAILED BUDGET JUSTIFICATION

Vanderbilt University Medical Center

PERSONNEL

(b)(6); (b)(3):7 U.S.C. § 8401 **Principal Investigator** (b)(4) **months)** (b)(6); (b)(3):7 U.S.C. § 8401 will direct projects principally involved with Specific AIM 2. (b)(6); (b)(3):7 U.S.C. § 8401 experience studying coronavirus replication and replicase nonstructural protein functions. He has published extensively on reverse genetics, replication and molecular biology of coronaviruses. (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401 For this project, the initial description of the GS-5734 inhibition of coronaviruses was initiated in the (b)(6); (b)(3):7 U.S.C. § 8401 and ongoing studies of the mechanism and data demonstrating resistance mutations were defined in the (b)(6); (b)(3):7 U.S.C. § 8401 will direct development of experiments with (b)(6); (b)(3):7 U.S.C. § 8401 and communicate with both Dr. Baric, other lab team members and with Gilead.

(b)(6); (b)(3):7 U.S.C. § 8401 **, Co-Investigator** (b)(4) **months).** (b)(6); (b)(3):7 U.S.C. § 8401 s a (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 with expertise in Molecular Virology, RNA viruses and Viral Diagnostics. (b)(6); (b)(3):7 U.S.C. § 8401 will directly perform experiments with SARS-CoV and MERS-CoV at BSL3 (b)(6); (b)(3):7 U.S.C. § 8401 will function as lab manager for all investigators at BSL3 and will work with (b)(6); (b)(3):7 U.S.C. § 8401 and participate in all conference calls, review of data. Dr. (b)(6); (b)(3):7 U.S.C. § 8401 ll manage all aspects of biosafety, biosecurity and shipping associated with this project.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) **months).** (b)(6); (b)(3):7 U.S.C. § 8401 is a (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 that has over 20 years of experience with coronavirus replication. Specifically (b)(6); (b)(3):7 will function in the design and generation of constructs, mutations and testing for toxicity in cell culture in parallel with BSL3 experiments. (b)(6); (b)(3):7 will carry out projects in coordination with (b)(6); (b)(3):7 U.S.C. § 8401 Particularly her expertise will be in the design and cloning of mutations, sequence analysis and review of results with Dr. (b)(6); (b)(3):7 U.S.C. § 8401

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) **months).** (b)(6); (b)(3):7 U.S.C. § 8401 is a (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 will coordinate preparation and maintenance of cells, cell culture, and reagents. (b)(6); (b)(3):7 U.S.C. § will perform RNA extractions and any model experiments at BSL2 for confirmation of phenotypes across the coronaviruses.

FRINGE BENEFITS: Fringe benefit calculations are derived from the current Vanderbilt University Medical Center guidelines.

LAB SUPPLIES: ($65,000)

Cell Culture Supplies, Serum and Media: ($15,000) A large amount of cell culture work is associated with the project, requiring media, serum and culturing flasks. Consequently, funds are requested for media, serum and related cell culture supplies to maintain Vero and Calu cells in culture, measuring virus growth kinetics, neutralization titers, and virus titers.

BSL3 Supplies, protective gear, disinfectants, decontamination: ($20,000) All in vitro transcription, electroporation, rescue and analysis of SARS-CoV and variants will be performed under strict BSL3 protocols. This will include extensive use of plasticware, tissue culture reagents, materials for plaque assays, and RNA isolation. BSL3 PPE (personal protective equipment) is also required for all work done at BSL3, as is an annual decontamination and recertification of the laboratory. Regular delivery of CO_2 for the incubators is also needed. In addition, supplies for analysis of RNA and protein at BSL2 as well as materials for shipping of samples between UNC and Vanderbilt are required.

Enzymes and Reagents: ($9,000) Generating mutations within the plasmids carrying fragments of the viral genomes will require the enzymes and reagents necessary for these molecular biology protocols. Assembling recombinant SARS-CoV and MERS-CoV requires large amounts restriction enzymes (e.g., BsmBI, etc.) and DNA ligase. DNA markers are needed for identifying appropriately sized bands and assembly intermediates

and full-length DNA products in gels; a critical step during the assembly of full-length cDNA clones. In addition, high quality T7 RNA polymerase is needed for driving production of full-length RNA transcripts for electroporation into susceptible cells and for the subsequent recovery of recombinant viruses. Chemicals used as RNA mutagens will also be needed

Genome sequencing and analysis: ($12,000) Sequencing of total cellular and virion RNA will be undertaken by conventional RT-PCR sequencing and by next-generation deep sequencing We will perform sequencing of genomes designed and recovered in the execution of the aims to confirm mutations and stability. Deep sequencing will be performed at Vanderbilt or UNC to test for diversity associated with experiments in Aim 2 both with virus mutants and with the effect of drug treatment.

Commercial cDNA synthesis and cloning: ($9,000) This is a critical category for generating the large number of viruses that carry mutations in nsp14 in SARS-CoV and MERS-CoV. This will also be a larger budget item early in the grant and will diminish as these viruses are generated and recovered. We will use commercial synthesis where it is more economical to have genome fragments synthesized compared to traditional mutagenesis.

OTHER EXPENSES: ($7,303)

Publications: ($2,000) Sufficient for 1-2 publications per year.

Repairs/maintenance: ($5,303) This will be for any required repairs of autoclave (BSL2, BSL3), prorated percent of maintenance contracts (25%) for spectrophotometer, and RT-qPCR, and centrifutes, replacement of small equipment (pipetmen, multipipettors), and other needed repair, maintenance and replacement.

TRAVEL: (3,000)
The budgeted amount will allow travel for 2-3 investigators to attend one meeting a year for presentation of scientific results and studies, such as American Society for Virology, the International Symposium on Plus-strand RNA Viruses and the International Nidovirus Symposium. These funds will also support two trips per year to UNC for direct meetings (low cost travel and lodging).

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		698,015.00
Section B, Other Personnel		175,470.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		873,485.00
Section C, Equipment		
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		361,515.00
1. Materials and Supplies	325,000.00	
2. Publication Costs	10,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	26,515.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		1,250,000.00
Section H, Indirect Costs		725,000.00
Section I, Total Direct and Indirect Costs (G + H)		1,975,000.00
Section J, Fee		

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator	(b)(4); (b)(6)				23,537.00	5,957.00	29,494.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **29,494.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			22,947.00	7,949.00	30,896.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Pathologist				4,250.00	1,076.00	5,326.00
1	Research Associate				24,240.00	8,397.00	32,637.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**68,859.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**98,353.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2017 **End Date*:** 05-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	85,661.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Per Diem Costs	9,880.00
9 . ARC support costs	16,900.00
10 . Histpathology Core Facility	5,000.00
Total Other Direct Costs	**117,441.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**218,794.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	55	218,794.00	120,337.00
		Total Indirect Costs	**120,337.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation; Arif Karim, Director; (214)767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**339,131.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	
	File Name: BudgetJustification1028523163.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator	(b)(4); (b)(6)				23,537.00	5,957.00	29,494.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **29,494.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			22,947.00	7,949.00	30,896.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Pathologist				4,250.00	1,076.00	5,326.00
1	Research Associate				24,240.00	8,397.00	32,637.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**68,859.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**98,353.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2018 **End Date*:** 05-31-2019 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	85,661.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Per Diem Costs	9,880.00
9 . ARC support costs	16,900.00
10 . Histpathology Core Facility	5,000.00
Total Other Direct Costs	**117,441.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**218,794.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	55	218,794.00	120,337.00
		Total Indirect Costs	**120,337.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation; Arif Karim, Director; (214)767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**339,131.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: BudgetJustification1028523163.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator	(b)(4); (b)(6)				23,537.00	5,957.00	29,494.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **29,494.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			22,947.00	7,949.00	30,896.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Pathologist				4,250.00	1,076.00	5,326.00
1	Research Associate				24,240.00	8,397.00	32,637.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**68,859.00**

Total Salary, Wages and Fringe Benefits (A+B) **98,353.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12254924
Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2019 **End Date*:** 05-31-2020 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	85,661.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Per Diem Costs	9,880.00
9 . ARC support costs	16,900.00
10 . Histpathology Core Facility	5,000.00
Total Other Direct Costs	**117,441.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**218,794.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	55	218,794.00	120,337.00
		Total Indirect Costs	**120,337.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation; Arif Karim, Director; (214)767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**339,131.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: BudgetJustification1028523163.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 . Dr.	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator	(b)(4); (b)(6)				23,537.00	5,957.00	29,494.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **29,494.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			22,947.00	7,949.00	30,896.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Pathologist				4,250.00	1,076.00	5,326.00
1	Research Associate				24,240.00	8,397.00	32,637.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**68,859.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**98,353.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12254924 Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2020 **End Date*:** 05-31-2021 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	85,661.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Per Diem Costs	4,940.00
9 . ARC support costs	8,450.00
10 . Histpathology Core Facility	5,000.00
Total Other Direct Costs	**104,051.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**205,404.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	55	205,404.00	112,972.00
		Total Indirect Costs	**112,972.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation; Arif Karim, Director; (214)767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**318,376.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: BudgetJustification1028523163.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 8007711490000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Texas Medical Branch

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 . Dr.	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator	(b)(4); (b)(6)				23,537.00	5,957.00	29,494.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **29,494.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			22,947.00	7,949.00	30,896.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Pathologist				4,250.00	1,076.00	5,326.00
1	Research Associate				24,240.00	8,397.00	32,637.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**68,859.00**

Total Salary, Wages and Fringe Benefits (A+B) **98,353.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 8007711490000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Texas Medical Branch

Start Date*: 06-01-2021 **End Date*:** 05-31-2022 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	18,161.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Per Diem Costs	4,940.00
9 . ARC support costs	8,450.00
10 . Histpathology Core Facility	5,000.00
Total Other Direct Costs	**36,551.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**137,904.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost Base	55	137,904.00	75,847.00
		Total Indirect Costs	**75,847.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation; Arif Karim, Director; (214)767-9861

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**213,751.00**

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: BudgetJustification1028523163.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

UTMB Budget Justification

Personnel

Key Personnel

(b)(6); (b)(3):7 U.S.C. § 8401 **Ph.D., PI of subcontract**, (b)(4) months). (b)(6); (b)(3):7 U.S.C. § 8401 will be responsible for the overall organization, coordination, and management of this project and personnel. (b)(6); (b)(3):7 will design the experiment and co-ordinate with the staff members and designated veterinarians to ensure all of the NHP studies and the subsequent downstream studies are well and timely executed. (b)(6); (b)(3):7 will be responsible for analyzing all of the data, prepare the reports for the PIs for this project, and assist other PIs with writing manuscripts for publications.

Other Personnel

(b)(6); (b)(3):7 U.S.C. § 8401 **Ph.D., designated pathologist** (b)(4) months). (b)(6); (b)(3):7 U.S.C. § 8401 is an experienced pathologist and has extensive experience working under BSL-3 and animal BSL-3 high containment laboratories. (b)(6); (b)(3):7 U.S.C. § will be responsible for evaluating the tissue histopathology.

(b)(6); (b)(3):7 U.S.C. § 8401 (b)(4) months). (b)(6); (b)(3):7 U.S.C. § 8401 has been well-trained for working under BSL-2 and BSL-3 laboratories. (b)(6); (b)(3):7 U.S.C. § has been working in (b)(6); (b)(3):7 U.S.C. § 8401 laboratory for the past 4+ years and will be in charge of determining the tissue distribution of viral infection by $TCID_{50}$- and qRT-PCR-based. (b)(6); (b)(3):7 will work closely with (b)(6); (b)(3):7 U.S.C. § 8401 and the "to-be-named Research Associate III or Research Scientist, as Lab manager, in BSL-3 laboratory. (b)(6); (b)(3):7 will also assist in maintaining the lab and keeping records related to the NHP studies. especially those for the transgenic colonies.

To Be Named Research Associate III/Research Scientist I (6 calendar months). The prospective candidate should be well-trained and experienced in working within BSL-3 as well as ABSL-3 containment laboratories. He or she will also serve as the lab manager and be the designated person in (b)(6); (b)(3):7 U.S.C. § 8401 laboratory for coordinating the group effort and carrying out downstream NHP studies with (b)(6); (b)(3):7 U.S.C. § 8401. The person will be responsible in maintaining and record-keeping for (b)(6); (b)(3):7 U.S.C. § 8401 laboratory.

Fringe benefit costs have been estimated as a percentage of institutional base salary according to the following schedule.

Institutional Base Salary	*Fringe Benefit Rate*
$ 1 – 68,594	34.64%
$ 68,595 – 83,500	27.51%
$ 83,501 – 118,499	25.31%
$118,500 – 142,499	22.87%
$142,500 – 181,999	20.78%
$182,000 – 264,999	18.81%
$265,000 +	14.30%

Materials and Supplies

Animal Purchase- NHPs will be purchased from a UTMB approved animal vendor and will be given a full health exam before shipment to UTMB. NHPs are estimated at $7,500 per animal, and a total of 36 animals will be purchased over the course of this project.

Year 1 (9 NHPs): $67,500
Year 2 (9 NHPs): $67,500
Year 3 (9 NHPs): $67,500
Year 4 (9 NHPs): $67,500

Animal Study Supplies- supplies needed for the NHP studies include items such as syringes, anesthetic, needles, PPE, blood collection tubes, and specimen jars. A total amount of **$3,163/year** is requested for supplies in this category.

General Lab Consumables ($10,000/year)- routine laboratory items are needed for the $TCID_{50}$ studies and the homogenization studies. Examples include PPE (gloves, respirators, cover gowns, etc.), pipette tips, culture tubes/plates, culture media, disinfectant, etc.

QPCR Reagents/Supplies ($5,000/year)- this category includes items such as PCR plates, primers/probes, and DNA purification kits.

Other Direct Costs

Histopathology Core Facility Fees ($5,000/year)- the Research Histopathology Core at UTMB will prepare, embed, stain, and analyze the histopathology samples for this project. This category also includes the reagents, stains, and supplies needed for sample processing.

Animal Per Diem Charges- NHPs will be housed in the (b)(3):7 U.S.C. § 8401 ABSL-3 facility for a study duration of approximately 24 days. Current rates are $45.74 per animal, per day. The table below summarizes the annual request for per diem fees.

Year 1	Year 2	Year 3	Year 4	Year 5
$9,880	$9,880	$9,880	$4,940	$4,940

**Study #4 will be occur across Years 4 and 5*

ARC Veterinary Support Charges- we will utilize the experience and expertise of the (b)(3):7 U.S.C. § Animal Resource Center to assist with NHP manipulations and procedures throughout this project. Current rates are $50/hr for vet tech support, and $100/hr for DVM support. The staff will assist with NHP vaccination, blood sampling, daily observations, challenge, and necropsy. For example, an estimated 185 hours of vet tech time and 77 hours of DVM time is estimated for each study involving 9 NHPs. The table below summarizes the annual request for ARC support fees.

Year 1	Year 2	Year 3	Year 4	Year 5
$16,900	$16,900	$16,900	$8,450*	$8,450*

**Study #4 will be occur across Years 4 and 5*

Travel

$3,000 is requested per year to fund the travel of the PI to a national or international scientific meeting to present research findings related to this project. All air travel will be limited to coach, and reimbursement of other related travel expenses will be limited to the prevailing standard per diems as designated in the federal travel regulations.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		147,470.00
Section B, Other Personnel		344,295.00
Total Number Other Personnel	15	
Total Salary, Wages and Fringe Benefits (A+B)		491,765.00
Section C, Equipment		
Section D, Travel		15,000.00
1. Domestic	15,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		492,925.00
1. Materials and Supplies	360,805.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	39,520.00	
9. Other 2	67,600.00	
10. Other 3	25,000.00	
Section G, Direct Costs (A thru F)		999,690.00
Section H, Indirect Costs		549,830.00
Section I, Total Direct and Indirect Costs (G + H)		1,549,520.00
Section J, Fee		

Funding Opportunity Number: RFA-AI-16-034 . Received Date: 2016-09-30T15:14:36.000-04:00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	1,241,271	966,654	966,654	953,264	885,764	**5,013,607**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	Specific_Aims1028716614.pdf
3. Research Strategy*	Research_Strategy1028821863.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	Protection_of_Human_Subjects1028716635.pdf
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	Inclusion_of_Women_Minorities1028523184.pdf
8. Inclusion of Children	Inclusion_of_Children1028523185.pdf
Other Research Plan Section	
9. Vertebrate Animals	Vertebrate_Animals1028716637.pdf
10. Select Agent Research	Select_Agent_Research1028716639.pdf
11. Multiple PD/PI Leadership Plan	MultiPI_Leadership_Plan1028716640.pdf
12. Consortium/Contractual Arrangements	Consortium_Agreements1028716643.pdf
13. Letters of Support	LOS_Gilead1028716646.pdf
14. Resource Sharing Plan(s)	Resource_Sharing_Plan1028716644.pdf
15. Authentication of Key Biological and/or Chemical Resources	authenticationof_Resources1028523241.pdf
Appendix	
16. Appendix	

2. Specific Aims. Zoonotic viruses, like coronaviruses (CoV), represent a continuous and growing threat to global public health because they unpredictably emerge, producing devastating outbreaks of pandemic disease. The CoVs are a genetically diverse family of RNA viruses infecting humans, mammals and birds. The inherently error prone viral RNA dependent RNA polymerase (RdRp) generates a genetically related yet diverse virus swarm (quasispecies) during replication, which promotes cross species transmission. In the 21st century, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged from zoonotic pools of viruses, causing severe disease in humans. Currently, MERS-CoV is endemic in camels in the Middle East with continuous new human infections. Although SARS-CoV is not currently a threat, several "prepandemic" SARS-like CoVs have been isolated from bats that replicate efficiently in human cells and are resistant to existing therapies. With the frequent overlap of human and wild animal ecologies, the potential for novel CoV emergence into humans is highly probable. Broad-spectrum CoV therapies that can control known human and zoonotic CoV infections would address an immediate unmet medical need and could counter future pandemic episodes.

Currently, there are no approved specific antiviral therapies for any human CoV infection. In partnership with Gilead Sciences, we have demonstrated that the nucleoside prodrug, GS-5734, is highly efficacious in inhibiting multiple human and zoonotic CoV in vitro and SARS-CoV in vivo. Preliminary studies argue that GS-5734 targets the RdRp, but the mechanism of action (MOA) for CoV remains unknown. We propose an academic-commercial partnership between UNC, Vanderbilt, and Gilead Sciences to accelerate the preclinical development of GS-5734 and provide proof of concept data necessary for IND licensure and the origination of a human clinical trial. *Specifically, we propose to define the pharmacokinetics, pharmacodynamics, resistance profile, spectrum of activity, and MOA of GS-5734 against SARS-CoV, MERS-CoV, zoonotic and less pathogenic human CoVs.* In the following aims, we will apply synthetic viral genome design, primary human cell cultures, in vivo imaging, improved animal models of human disease with clinically applicable endpoints, and state-of-the-art expertise in preclinical pharmaceutical development to develop and evaluate GS-5734.

Aim 1: Pharmacokinetics and Pharmacodynamics of GS-5734. In **part 1**, we synthetically reconstruct group 2D CoV to comprehensively assess spectrum across the CoV family. Human cell uptake and metabolism of prodrug to the active triphosphate (TP) form directly governs the magnitude of antiviral effect, therefore, **part 2**, will determine if antiviral effect and drug metabolism are equivalent in various primary cells targeted by SARS- and MERS-CoV (i.e., lung endothelial, type II pneumocyte, T-cells, etc.) through measurement of TP levels, virus replication and toxicity. Studies assessing the in vivo efficacy of GS-5734 against MERS-CoV have been hampered by genetic differences between mouse and human. In **part 3**, we will create a transgenic mouse deficient in serum esterase, *Ces1c*, and expressing a permissive form of the MERS-CoV receptor, DPP4, for evaluating antiviral efficacy against MERS-CoV in vivo. We will assess treatment efficacy in young and aged mouse models of SARS- and MERS-CoV disease. In **part 4**, we will assess antiviral efficacy of GS-5734 in non-human primate models of SARS- and MERS-CoV pathogenesis.

Aim 2: Defining Resistance to GS-5734 and Impact on Replication, Pathogenesis and Treatment. Passage of the murine hepatitis CoV (MHV) in the presence of GS-5734 parent drug generated mutations in the RdRp; however, the pathways to resistance for SARS- or MERS-CoV remain unknown. In this aim, *we will determine if resistance is mediated by mutations in nsp12, nsp14-ExoN, or other replicase proteins and test the impact of resistance mutations on virus replication, RNA synthesis, and fitness in vitro and pathogenesis in vivo*. In **part 1**, we will passage MERS-CoV and SARS-CoV in the presence of GS-5734 in continuous and primary human airway cells, and in wild-type and immunodeficient animals to compare resistance pathways across viruses and biological systems. In **part 2**, we will determine the effect of passage-selected reverse-engineered GS-5734 resistance mutations on replication fidelity, viral RNA synthesis, and competitive fitness as compared to wild-type parental virus. In **part 3**, we will define the effect of resistance mutations on viral replication, pathogenesis, and treatment in murine models of SARS-CoV and MERS-CoV pathogenesis.

Aim 3: Defining the Mechanism of Action of GS-5734. We hypothesize that GS-5734 fosters the generation of incomplete, partial, or mutated viral RNA, leading to altered antiviral innate immune responses, loss of viral fitness, and/or attenuated viral pathogenesis. **In part 1**, we will determine the effect of GS-5734 on SARS- and MERS-CoV viral RNA synthesis, sequence diversity, and the host innate immune response through deep RNA-sequencing of drug-treated infected human airway epithelial cells. In **part 2**, we will determine the effect of GS-5734 on viral RNA synthesis, sequence diversity, and innate immune response in infected WT and immune deficient mice. In **part 3**, we will use single-molecule RNA FISH to determine the effect of drug on viral RNA replication and the innate immune response at single-cell resolution. These studies should reveal if the antiviral MOA is a result of direct effects on viral RNA replication and/or alteration of antiviral immunity.

3. RESEARCH STRATEGY

3.1 SIGNIFICANCE AND IMPACT. New strategies are needed to protect against emerging, highly pathogenic zoonotic coronaviruses (CoVs), whose genetic diversity can render vaccines and therapeutics ineffective [1, 2]. Currently, there are no FDA approved therapeutics for treating human or zoonotic CoVs. Through our integrated academic/industry partnership, we have identified a lead molecule nucleoside analog prodrug, GS-5734 (Gilead Sciences) that is highly efficacious against the Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and multiple other zoonotic and human CoVs. The goal of our program is to accelerate the preclinical development of GS-5734 to support IND licensure for MERS-CoV and to accumulate key pre-clinical data for the continued progression towards human clinical trials. Our established and productive collaboration between UNC, Vanderbilt University Medical Center (VUMC), and Gilead Sciences integrates leading edge molecular virology, recombinant viral genetics, primary human cell models, metabolic and pharmacokinetic (PK) analysis, and murine and primate models of human coronavirus disease. Together, we will define: 1) efficacy, activity breadth, and drug metabolism in multiple primary human cells, 2) PK and efficacy in small and large animal models, 3) mechanism of action (MOA) and resistance profile, and 4) impact of resistance on treatment, fitness and disease.

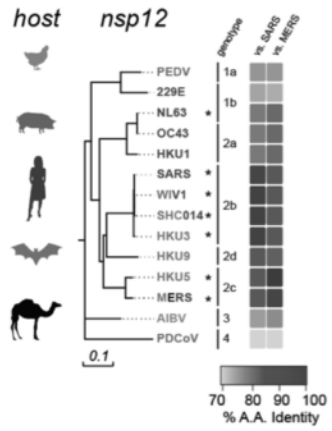

Figure 1. CoV RNA-dependent RNA polymerase (nsp12) is conserved among the genetically diverse family of CoVs. Neighbor-joining tree created with representative CoV from all four genogroups show high similarity among nsp12 (RdRp). Text colors of virus strains in trees correspond to host species on the left. Asterisks indicate strains for which we have built molecular clones.

Coronavirus Pandemic Potential, Endemic Human Disease and the Need for Therapeutics. The CoV are a genetically diverse family of RNA viruses infecting invertebrates, birds and mammals (**Fig. 1**), many of which have demonstrated zoonosis capacity or potential [3-6]. Even the endemic human CoVs (HCoV-NL63, HCoV-OC43, HCoV-229E and HCoV-HKU1) were once emergent zoonosis and likely originated from bats, cattle and/or rodents ~100-800 years ago[4]. While infection with endemic HCoVs typically cause the common cold, infection of young children, adults and the elderly can lead to more severe disease, including asthma/chronic obstructive pulmonary disease (COPD) exacerbations, pneumonia, and death [7-10].

With the appearance of SARS- and MERS-CoV in humans, the emergence potential of CoVs and their ability to cause severe human disease was confirmed [3-6]. In 2002, SARS-CoV emerged from bats in Guangdong China, causing over 8000 cases with 10-50% mortality as a function of increasing age [11-15]. While SARS-CoV is not a current threat, several SARS-like bat CoVs can bind and enter human cells via the SARS-CoV receptor (ACE2), replicate efficiently in primary human airway cells, and are resistant to existing therapeutic antibodies and vaccines [1, 2, 16, 17]. In 2012, MERS-CoV was discovered to have evolved from bats to infect humans via intermediate camel hosts. MERS-CoV continues to cause illness and death, with over 1800 cases in ~27 countries and ~36% mortality [3, 18]. Serologic studies in the Kingdom of Saudi Arabia and Kenya demonstrate MERS-CoV endemicity with an estimated 45,000 seropositive individuals, and recent models argue that severe cases are 3-fold more common than previously thought [19, 20]. Like SARS-CoV, MERS-CoV-like viruses have been isolated from bats in China and elsewhere [21]. With increasing overlap of human and wild animal ecologies, the potential for future severe zoonotic CoV emergence is high. Currently, there are no approved specific antiviral therapies to treat any human CoV infection. Attempts to treat both SARS- and MERS-CoV patients with approved antivirals and immune modulators have failed in randomized controlled trials [22-29], and clinical development of effective CoV-specific antivirals has remained elusive [28].

Coronavirus Polymerase and Proofreading Exonuclease in High-Fidelity RNA Synthesis. CoVs encode the largest known RNA genomes (28 to 32 kb). Following cell entry, the CoV genome RNA is translated to yield 16 nonstructural proteins, of which nsp7-14 are proposed to form a multiprotein replication/transcription complex responsible for genome replication and subgenomic RNA synthesis. CoVs are unique among RNA viruses because they not only encode an RNA-dependent RNA polymerase (nsp12-RdRp) but also encode a DE-D-Dh superfamily 3'-5' exoribonuclease in nonstructural protein 14 (nsp14-ExoN). The [redacted] and Baric Labs have shown that nsp14-ExoN is required for high-fidelity replication, and is likely the first identified RNA-dependent RNA proofreading enzyme [30-33]. Further, we have shown that murine hepatitis virus (MHV) and SARS-CoV are resistant to multiple mutagens (ribavirin, 5-FU, 5-azacytidine), while nsp14 ExoN-inactivated (ExoN(-)) mutants are exquisitely sensitive to these drugs, indicating that the ExoN activity is critical for nucleoside selectivity [34].

(b)(6); (b)(3):7 U.S.C. § 8401

Potential Mechanism of Action of Nucleoside Prodrug GS-5734 Against CoVs. The nsp12-RdRp is one of the most highly conserved CoV proteins across and within genogroups (~70-90% amino acid identity), making this protein a very appealing drug target (**Fig. 1**). In collaboration with Gilead Sciences, we identified a monophosphoramidate prodrug, GS-5734, that was highly active against MERS-CoV, SARS-CoV and MHV with EC_{50} values of 0.03, 0.05 and 0.03 μM (**Fig. 2a,b**), respectively, and potency of 4-5 log_{10} reduction in virus titer. This is the first nucleoside analog or mutagen demonstrating robust inhibition of the CoV RdRp while resisting the activity of nsp14-ExoN proofreading suggesting a potentially unique MOA (**Fig. 2c,d**). GS-5734 was recently reported by Warren et. al to be efficacious against Ebola virus in non-human primates and biochemical data with the RdRp of respiratory syncytial virus (RSV) demonstrated a MOA through RNA chain termination[35]. Passage of MHV in the presence of parent adenosine nucleoside analog, GS-441524, selected for partial resistance and two amino acid changes in the nsp12-RdRp (F476L and V553L). Introduction of these mutations into SARS-CoV using reverse genetics conferred partial resistance to GS-5734 similar to that seen in MHV (**Fig. 2c,d**). We do not yet know whether GS-5734 functions as a direct polymerase inhibitor, chain terminator, or mutagen for CoV or if multiple genetic pathways mediate resistance. In **Aims 2 and 3**, we test the hypothesis that GS-5734 acts as a polymerase inhibitor of CoV and that mutation of the nsp12-RdRp and/or other non-structural proteins mediate resistance.

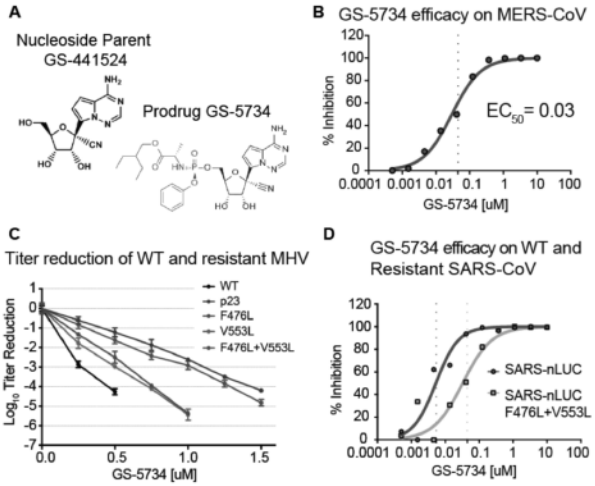

Figure 2. GS-5734 inhibition of CoV replication, resistance and possible mechanism. **A)** Structure of nucleoside analog GS-441524 and prodrug GS-5734. **B)** Inhibition of MERS-CoV replication (EC_{50} < 0.03 μM). **C)** Mutations selected in the nsp12-RdRp (F476L and V553L) are 100% conserved across all CoVs and confer partial resistance to GS-5734. **D)** SARS-CoV encoding V553L/F476L results in 5-fold increase in EC_{50} for GS-5734 in vitro.

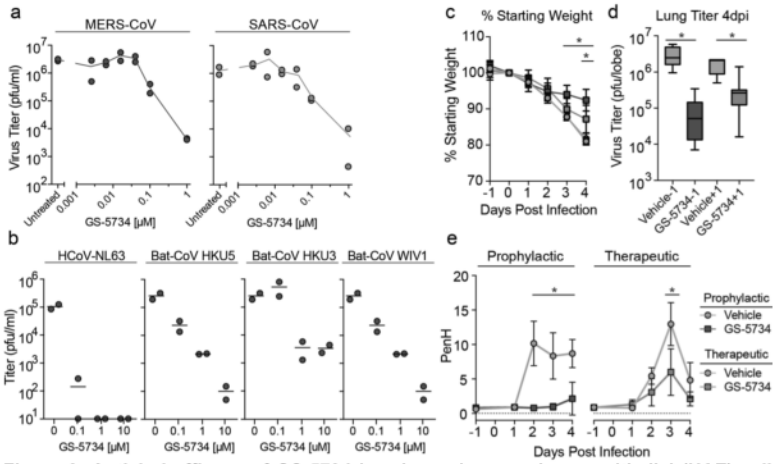

Figure 3: Antiviral efficacy of GS-5734 in primary human airway epithelial (HAE) cell cultures and mice. A) MERS- and SARS-CoV-infected HAE (MOI = 0.5) treated with increasing doses of GS-5734. B) HAE treated and infected as in Panel A. Group 1 human CoV NL63, group 2C MERS-like bat CoV HKU5, divergent group 2b bat CoV HKU3, and SARS-like prepandemic bat CoVs WIV1. Vehicle or GS-5734 (25 mg/kg) was administered twice daily beginning either day -1 or day +1 post-infection. C) Percent of starting weight demonstrating protection from weight loss with GS-5734 treatment. D) SARS-CoV titer in the lung is reduced with GS-5734 treatment. E) Pulmonary function as measured by whole-body plethysmography. Penh is a measure of airway obstruction.

Broad Activity of GS-5734 Against SARS-CoV, MERS-CoV and Prepandemic Zoonotic CoVs In Vitro and In Vivo. Using primary human airway epithelial (HAE) cell cultures, we have shown that GS-5734 is effective against MERS- and SARS-CoV (**Fig. 3a**), as well as group 1 human endemic HCoV-NL63, group 2B SARS-like bat CoV (HKU3), MERS-like group 2C bat CoV (HKU5), and group 2B prepandemic bat CoV (WIV1) (**Fig. 3b**). GS-5734 abrogates SARS-CoV disease in a mouse model of lethal SARS-CoV infection, protecting against replication, clinical disease, respiratory dysfunction, and pathology (**Fig. 3c-e**). The pharmacokinetic profile of GS-5734 in mice suggests that doses with substantial antiviral effects are well tolerated in humans. **Aim 1** will detail the pharmacokinetics and efficacy in animal and primate models. **Aims 2 and 3** will test the impact of resistance on replication, disease, treatment and pathogenesis in vitro and in vivo.

Coronavirus Genetics and Testing of GS-5734. Our groups have pioneered synthetic genome design and reverse genetics systems for CoVs that facilitate the generation of recombinant high-risk emerging CoVs and mapping of drug-resistance alleles. We have recovered a variety of group 1 and 2 zoonotic and human CoVs, including MHV, SARS-CoV, MERS-CoV, HCoV-NL63, HKU5, HKU3, WIV1, SCH014 and others[1, 2, 36-41]. In this proposal, we will assemble a panel of viruses representative of family-wide CoV genetic diversity, including other endemic human strains and uncharacterized group 2D strains, to directly test whether antiviral activity of GS-5734 is maintained across divergent CoVs and cell types, and to identify common pathways of function and resistance. We hypothesize that GS-5734 will be efficacious against current human and zoonotic

CoVs, and emerging CoVs of the future. Thus, we anticipate clinical utility beyond the immediate need to treat MERS-CoV infections.

3.2 INNOVATION. Advances in Coronavirus Therapeutic Evaluation. In collaboration with Gilead Sciences, we will apply a decade of experience studying virus replication in human primary lung cell models to determine if uptake, metabolism and antiviral efficacy are similar in the array of cells targeted by SARS- and MERS-CoV in vivo. Uniform biodistribution and metabolism of drug in cells targeted by virus will ensure maximal antiviral effect in humans. We also will employ state-of-the-art technologies to quantify in vivo efficacy and amelioration of clinical disease in mouse models of SARS- and MERS-CoV pathogenesis. We will utilize in vivo bioluminescent imaging (BLI) to monitor reporter-virus replication in live animal cohorts. In vivo BLI provides richer longitudinal metrics of virus replication and spread in real-time without the need to sacrifice animals, and has been successfully used to evaluate influenza and RSV therapeutics[42, 43]. To complement our analysis of lung virus titer, pathology, and virus antigen, we measure pulmonary function via whole-body plethysmography, 22-parameter complete blood count (CBC) via Vetscan HM5c, and inflammatory biomarkers via BioPlex. Lastly, we will collaborate with (b)(6); (b)(3):7 U.S.C. § 8401 at University of Texas Medical Branch to assess antiviral efficacy in non-human primate models of CoV disease.

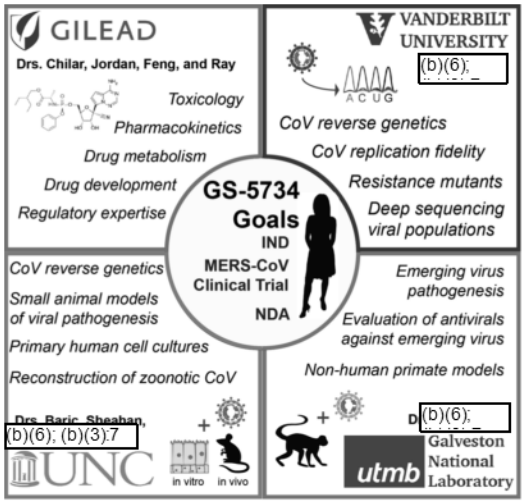

Figure 4. Research team structure, expertise, milestones and goals for the progression of GS-5734.

Team Integration and Innovation. This program extends an ongoing, highly interactive collaboration to accelerate the pre-clinical development of GS-5734, promote IND licensure for the MERS-CoV indication, and inform future human clinical trials. Success of this work will bring GS-5734 forward as the first and only specific antiviral targeting CoV in humans and support additional indications, including Ebola. We will achieve the proposed aims and objectives using complementary expertise of groups at UNC, VUMC, and Gilead Sciences (**Fig. 4**), resulting in a comprehensive preclinical package of in vitro, in vivo, genetic and mechanistic data. The **Baric Lab** (UNC) has a long history using reverse genetics, metagenomics and synthetic genome design to recover recombinant viruses; primary human airway cells to study emerging virus-host interactions and replication; and development of robust small animal models of human disease[1, 36, 39, 40, 44-48]. **Dr. Sheahan** (UNC) has over a decade of experience in academic translational research with CoV and HCV and also has crucial industrial preclinical antiviral development experience gained while at GlaxoSmithKline[49-56]. His understanding of the academic and industrial enterprise has proven essential to the success of the current collaboration with Gilead Sciences. The (b)(6); (b)(3):7 U.S.C. § 8401 (VUMC) discovered ExoN proofreading and polymerase function and is expert in using RNAseq to measure replication fidelity[30-32, 34, 57]. (b)(6); (b)(3):7 U.S.C. § 8401 UTMB) has a rich history of CoV animal model development, and in vivo vaccine and therapeutic studies[58-60]. **Gilead Sciences** is a leader in antiviral drug design and has commercialized products to treat viral diseases such as HIV and HCV[61, 62]. Gilead Sciences' expertise in pharmacokinetics, medicinal chemistry, preclinical development and the programmatic transition to human trials will ensure achievement of realistic milestones and timelines.

3.3 APPROACH

3.A. Specific Aim 1. Pharmacokinetics and Pharmacodynamics of GS-5734.

Rationale: Knowledge of the uptake and metabolism of prodrug to the active triphosphate (TP) form by all permissive cell types targeted by a virus in vivo is critical to understand the potential antiviral effect and capacity to ameliorate clinical disease. We have shown that MERS-CoV infects primary human HAE cells, type II pneumocytes, lung fibroblasts, and lung microvascular endothelial cells (**Fig. 5a**), whose damage can lead to diffuse alveolar damage, intra-alveolar edema, and compromised pulmonary function in vivo. Additionally, we find that MERS-CoV infects human $CD4^+$ T cell subpopulations (**Fig. 5b**), which may have dire consequences for induction of adaptive immunity. We have demonstrated GS-5734 efficacy in HAE **(Fig. 3).** Demonstrating potent antiviral activity of GS-5734 in these critical cell types will support the preclinical data package. In this Aim we will: 1) Evaluate the activity, metabolism and toxicity of GS-5734 in the primary cells targeted by MERS-CoV in vivo, 2) Assess drug efficacy against SARS-CoV in a subset of permissive human cells, followed by other zoonotic and prepandemic bat CoVs, 3) Since both SARS- and MERS-CoV cause acute respiratory distress syndrome (ARDS), a lethal end stage lung disease where age is a critical risk factor for disease

severity, we will assess efficacy in young and aged mouse models of SARS- and MERS-CoV pathogenesis which recapitulate increased disease severity with age [63, 64], 4) In collaboration with investigators at UTMB (b)(6); (b)(3):7 U.S.C. § 8401 we will assess the therapeutic activity of GS-5734 in non-human primates infected with MERS-CoV and then both prophylactic and therapeutic efficacy with SARS-CoV, 5) We will work closely with Gilead Sciences to assess pharmacokinetics, toxicity and metabolism in mouse and non-human primate models utilized for efficacy testing. Primarily, the Baric, Sheahan and (b)(6); (b)(3):7 U.S.C. § laboratories partner with Gilead in Aim 1.

3.A.1. Isolation of Recombinant Viruses. Our group has recovered representative group 1 and 2 emerging (MERS-CoV, SARS-CoV), zoonotic bat (HKU3, HKU5), prepandemic bat (WIV1, SHC014) and endemic human coronavirus strains (HCoV-NL63). We have not tested GS-5734 activity against any group 2D coronavirus. Using our well-established approach, we will synthesize two distant group 2D bat coronavirus genomes (HKU9-2, Ky24), replacing the S glycoprotein ectodomain with that of either the mouse-adapted SARS-CoV or MERS-CoV, allowing for efficient replication in human primary cells, and pathogenesis in mice[36]. This alteration of HKU9-2 and Ky24 host range and pathogenesis capacity in mice will likely constitute a gain-of-function (GOF). If this is problematic, we will utilize the bat CoV WIV1 S gene to facilitate the study of group 2D viruses in human cells.

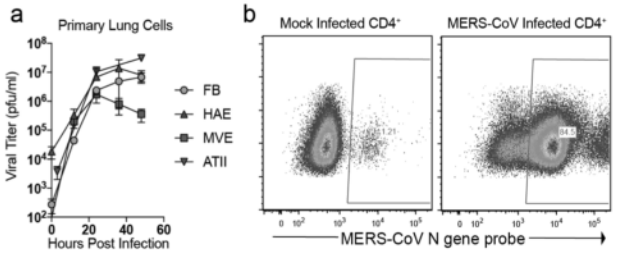

Figure 5: Primary lung and leukocyte infection with MERS-CoV. a) Infectious virus production in primary human lung fibroblasts (FB), human airway epithelial cell cultures (HAE), human lung microvascular endothelial cells (MVE) and alveolar type II cells infected with MERS-CoV (MOI = 0.5). b) Human CD4+ T-cell infection with MERS-CoV. Positively selected CD4+ T-cells infected with MERS-CoV (MOI = 1.5) stained for MERS-CoV N mRNA by branched DNA FISH at 24hpi and analyzed via flow cytometry.

3.A.2. GS-5734 Antiviral Activity in Primary Human Cells.

i. Lung Airway and Related Cells. Dr (b)(6); (b)(3):7 U.S.C. § 8401, an expert in the cultivation of primary human lung cell types, will prepare primary HAE cultures, type II pneumocytes, lung fibroblasts, and lung endothelial cells from three separate human donors[1, 2, 39, 65]. Although we have demonstrated efficacy of GS-5734 for MERS-CoV, SARS-CoV and select other CoV in HAE cultures (**Fig. 3a,b**), we propose to extend these HAE studies to other HCoVs - OC43, HKU1 and 229E - as well as group 2D bat CoV from Section 3.A.1. MERS-CoV and SARS-CoV also infect type II pneumocytes but only MERS-CoV infects primary lung fibroblasts and microvascular endothelial cells. These cells are critical to alveolar function and integrity and GS-5734 mediated protection of the cells will provide an invaluable insight in the potential mitigation severe lung pathologies associated with destruction of alveoli compartment. To this end, primary cells noted above will be infected with MERS-CoV expressing nanoluciferase (MERS-nLuc) and treated with a dose range of GS-5734 (0.01-10 μM) [1, 39]. At 48 hours post infection (hpi), virus replication will be quantified by luminometer and real-time RT-PCR. Similar studies will be performed in the subset of cells targeted by SARS-CoV. These studies will confirm drug efficacy in key primary cell types that mediate severe disease by SARS- and MERS-CoV and also thoroughly demonstrate GS-5734 exerts family-wide broad-spectrum anti-CoV activity in primary human cells.

ii. Primary Immune Cells. Severe cases of primary MERS-CoV infection are associated with immune suppression as evidenced by significantly reduced or delayed antibody responses[66]. Mechanisms of immune suppression are unknown. Primary human $CD4^+$ T cells are infected by MERS-CoV (**Fig. 5b**) and alteration of their helper function due to direct infection by MERS-CoV may potentially explain the observed dysregulation of humoral immunity. Thus, the antiviral effect of GS-5734 exerted in T-cells may reverse immune suppression and generate a more protective humoral response. To evaluate antiviral efficacy of GS-5734 in primary immune cells, we will isolate $CD4^+$ T cells from donated human peripheral blood mononuclear cells (PBMCs) by magnetic positive selection and infect 2 x 10^6 cells/well with MERS-CoV in the presence increasing doses of GS-5734. At 36 hpi, virus production will be quantitated by plaque assay and infection frequency by flow cytometry using PrimeFlow to measure MERS-CoV nucleocapsid (N) RNA [67]. In cells from multiple different donors, we see high virus titers (>10^5 pfu/ml) and >80% of $CD4^+$ T cells staining positive for MERS-CoV RNA (**Fig. 5b**). Since similar compounds targeting HIV are effectively transported and metabolized in T-cells, we anticipate GS-5734 will diminish MERS-CoV replication in this compartment[62].

iii. In Vitro Toxicity and Metabolism of GS-5734. Concurrently, we will assess cytotoxicity in uninfected primary cells treated with a dose response of GS-5734 similar to that utilized in the antiviral assays noted above. At 48 hours post-treatment (hpt), cytotoxicity will be measured via Cell-Titer Glo assay (Promega), and transcripts guiding apoptosis will be measured in total RNA by TaqMan based RT-PCR assays. To study in vitro metabolism of GS-5734, primary cells will be incubated with 1 μM GS-5734 for 48 hours at 37 ˚C. At 2, 8,

24, 36 and 48 hpt, cells will be washed with ice-cold saline and scraped into ice-cold 70% methanol containing 2-chloro-adenosine-5'-triphosphate (Sigma-Aldrich,) as an internal standard. Samples will be shipped overnight at -20 ˚C to Gilead Sciences for analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

3.A.3. GS-5734 Prophylactic and Therapeutic Activity Against MERS-CoV In Vivo.

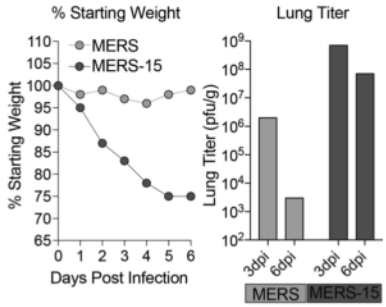

Figure 6: MERS-15 pathogenesis in DPP4 288/330$^{+/+}$ mice. Passage of MERS in vivo yielded a more virulent virus, MERS-15, which causes dramatic weight loss and high-titer lung replication.

i. MERS-CoV Mouse Model. Rodent orthologs of the human receptor, dipeptidyl peptidase 4 (DPP4) do not support MERS-CoV infection preventing small animal model development[68]. Since we and others demonstrated that transgenic overexpression of human DPP4 in mice led to death from fatal viral encephalitis post MERS-CoV infection, rather than severe lung disease seen in human patients, we used CRISPR/Cas9 to introduce two human codons at positions 288 and 330 of the mouse DPP4 receptor (i.e. mDPP4 288/330$^{+/+}$ mice, Cockrell et. al, in press)[59, 63, 69]. With native mDPP4 expression but human DPP4 alleles at 288/330, we show replication of MERS-CoV primarily in the lung without neurological infiltration. Following GOF approval by the NIH, MERS-CoV was passaged in vivo, giving rise to a mouse-adapted virus (MERS-15) that causes ~30% weight loss, significant reductions in pulmonary function, targeting of airway epithelium, type II pneumocytes and endothelium, and an ARDS-like pathology that is uniformly lethal day 6 postinfection (**Fig. 6**). Unlike humans, mice express a secreted carboxylesterase 1c (*Ces1c*), which rapidly metabolizes GS-5734 in the blood before adequate distribution to target tissues. To circumvent this, we utilized mice deficient in *Ces1c*$^{-/-}$ to assess GS-5734 efficacy against SARS-CoV in vivo. Importantly, SARS-CoV pathogenesis was similar in wild-type C57BL/6 and *Ces1c*$^{-/-}$ mice. In collabroation with Gilead Sciences and Jackson Laboratories (JAX), we are generating a *Ces1c*$^{-/-}$ and mDPP4 288/330$^{+/+}$ (*Ces1c*$^{-/-}$/288/330$^{+/+}$) mouse colony to facilate comprehensive evaluation of GS-5734 for MERS-CoV. After successful crossing of the two mouse strains, Gilead is overseeing the breeding at JAX and sufficient *Ces1c*$^{-/-}$/288/330$^{+/+}$ mice will be available in early 2017. After confirming MERS-CoV pathogeneis is similar in 288/330$^{+/+}$ and *Ces1c*$^{-/-}$/288/330$^{+/+}$mice, we will evaluate the prophylactic and therapeutic efficacy of GS-5734 in this model.

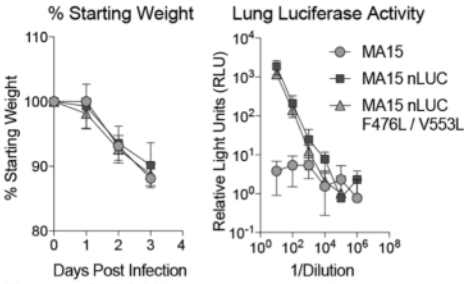

Figure 7. SARS-MA15 reporter and resistant mutant reporter viruses are fully pathogenic in vivo and express luciferase in the lungs of infected mice.

ii. Prophylactic and Therapeutic Treatments. Prophylactic efficacy studies will first be performed in young (20 week) and then in aged mice (1-1.5 yr), the latter model capturing the increased vulnerability and severe disease phenotypes seen in aged human patients [36, 52, 70, 71]. With the aid of in vivo imaging of virus replication in live animals, and multiple clinically applicable metrics (complete blood count, pulmonary function, inflammatory biomarkers, etc.), our proposed in vivo efficacy studies improve upon our previous work with GS-5734 and SARS-CoV. Briefly, 25mg/kg GS-5734 (n=6) or vehicle (n=6) will be administered subcutaneously twice daily (BID) beginning 1 day prior to infection, using 20 and then ~60 week old *Ces1c*$^{-/-}$/288/330$^{+/+}$ mice infected with MERS-15 expressing nano-lucferase (MERS-15 nLUC). Weight loss, virus replication via IVIS Lumina III, respiratory function (whole body plethysmography) and morbidity will be evaluated daily through 6dpi.

A parrallel cohort will be similarly treated and infected (vehicle n = 12, GS-5734 n = 12) and half the animals will be sacrifced on days 3 and 6 days post infection for complete blood count via Vetscan HM5c, lung pathology viral antigen staining to determine viral tropism (e.g. airway epithelium, lung endothelial cells, type II pneumocytes), viral titer determination via plaque assay, viral RNA quantitation by RT-PCR, and serum cytokine analysis via BioPlex. As SARS-CoV nLUC is fully pathogenic in mice (**Fig. 7**), we will perform pilots to ensure that MERS15 nLUC is also fully pathogenic before initiating these experiments. Luciferase expression, whose levels parallel levels of replication, will be detected by the IVIS Lumina III as emitted light immediately after injection of luciferase substrate. In fullfillment of 3Rs (reduction, refinement, replacement) principle that guides humane animal research, this technology is exquisitely sensitive and allows for noninvasive repeated longitudinal measures eliminating the need to sacrifice multiple cohorts of mice, a critical barrier in performing studies in aged animals. Since GS-5734 diminished SARS-CoV replication and disease and improved pulmonary function in mice (**Fig. 3**), we anticipate similar results the above MERS-CoV efficacy studies. To assess spectrum and breadth of activity in aged animals, similar studies will be performed with SARS-CoV, group 2b (HKU3) and 2c (HKU5) bat CoV mouse adapted strains.

If prophylactic studies are successful, we will test if therapeutic administration of GS-5734 can prevent lethal/severe disease outcomes first in young and then in aged mice. Briefly, 25mg/kg GS-5734 or vehicle will be administered subcutaneously twice daily (BID) beginning either 1 day prior, 1 day post or two days post MERS-15 nLUC infection. For each treatment group and each time of addition, 6 mice will be infected (i.e. begin treatment on -1dpi, n=6 vehicle, n=6 GS-5734). Experimental endpoints identicial to those noted in prophylactic studies will be evaluated with therapeutic treatment. We anticipate treatment 1 dpi will significantly decrease rates of disease and death, but treatment beginning 2dpi may not confer protection due to the rapid progression of disease. To assess spectrum in aged animals, similar studies will be performed with SARS-CoV, group 2b (HKU3) and 2c (HKU5) bat CoV mouse adapted strains.

3.A.4. In vivo Pharmacokinetic Analysis and Metabolism of GS-5734. Gilead Sciences has already completed a thorough pharmacokinetic (PK) analysis of GS-5734 in young *Ces1c*$^{-/-}$ mice (**Fig. 8**) but has not yet assessed PK in aged mice. With the creation of a new transgenic strain for the study of efficacy with MERS-CoV, we will reassess the PK profile of GS-5734 in *Ces1c*$^{-/-}$/288/330$^{+/+}$ in collaboration with Gilead Sciences. While we do not anticipate the PK of GS-5734 to differ with mouse genotype or age, it is possible those factors will affect bio-distribution and/or metabolism given how host genetics and age can alter host gene expression patterns [71, 72].

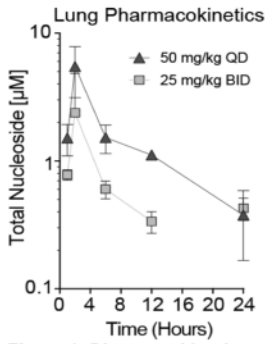

Figure 8. Pharmacokinetics of GS-5734 in *Ces1c*$^{-/-}$ mice. Subcutaneous 50mg/kg once or 25mg/kg twice daily. Total nucleoside was measured in the lungs via LC/MS.

3.A.5. In vivo Efficacy of GS-5734 in NHP models of MERS-CoV and SARS-CoV. Due to the genetic, physiologic, immunologic, and metabolic similarities with humans, antiviral efficacy studies in non-human primates (NHP) facilitate accurate dose prediction and toxicity assessment in genetically outbred higher species. Gilead Sciences has recently demonstrated prophylactic GS-5734 diminished MERS-CoV replication and disease in rhesus macaques. Interestingly, renal toxicity was observed but only in infected and treated animals (**See Gilead Sciences Letter of Support**). In collaboration with Gilead Sciences and (b)(6); (b)(3):7 U.S.C. § 8401at UTMB and the Galveston National Laboratory (GNL), we aim to continue these NHP studies first with therapeutic efficacy studies with MERS-CoV in rhesus macaques and then prophylactic and therapeutic efficacy studies with SARS-CoV in African green monkeys.

i. Therapeutic Efficacy Studies with MERS-CoV in Rhesus Macaques. Healthy control (n = 3) and treated (n = 6) groups of adult male and female rhesus macaques will be anaesthetized and infected with 7 x 10^6 $TCID_{50}$/each MERS-CoV (EMC-2012 isolate) through a combined intratracheal (i.t.), intranasal (i.n.), and oral/ocular (o/o) route as described[73]. Once daily intravenous treatment with vehicle (control) or 5mg/kg GS-5734 (treated) will begin 8hpi. Temperature will be monitored continuously via implanted IPTT-300 (Biomedic) probes. Daily swabs (nasal and oral) and blood draws will be taken to monitor virus shedding/viremia via plaque assay/real-time RT-PCR and to measure alterations in blood cell populations/chemistry (HemaVet 950FS+ laser-based hematology analyzer, Drew Scientific). At the peak of viral infection and disease (3dpi), animals will undergo CT scan to image pulmonary abnormalities (i.e. ground glass opacity, consolidation, interstitial pulmonary edema, etc.). Animals will then be euthanized and various organs and tissues will be collected to assess virus replication and pathology.

ii. Therapeutic and Prophylactic Studies with SARS-CoV in African Green Monkeys. Healthy control (n=3), prophylactic (n=6) and therapeutic (n=6) male and female African green monkeys will be anaesthetized and infected with 7 x 10^6 $TCID_{50}$/each SARS-CoV (Urbani strain) through a combined i.t, i.n., and o/o route. At 8hr before (prophylactic) or 8hr after (therapeutic), once daily intravenous treatment will begin with vehicle or 5mg/kg GS-5734 (treated). Daily monitoring, endpoints and analysis will be as described for MERS-CoV.

3.A.6. Expected Outcomes and Alternatives. Our groups are experienced in all of the procedures described in Aim 1, thus we do not anticipate any serious problems with the approach. We expect GS-5734 treatment to significantly reduce MERS- and SARS-CoV replication and pathology with prophylactic and therapeutic treatment, both in mice and NHPs. The world-class scientific and veterinary staff at UTMB/GNL have an excellent track record evaluating therapeutics against emerging human pathogens including select agents like Ebola, Marburg, and Nipah viruses in NHPs. Moreover, the NHP models described above are well established for CoV pathogenesis[73-75]. While renal toxicity was observed in MERS-CoV and GS-5734 treated NHP at the highest dose (10mg/kg), this was reduced in animals that received lower drug doses (5 mg/kg). The reversibility of this treatment-associated toxicity is not known and some SARS-CoV studies may be deprioritized to investigate this if it becomes apparent renal toxicity investigation is essential for development.

To determine if renal toxicity is reversible, an additional arm can be added to the MERS-CoV study above. Animals will be discontinued GS-5734 treatment after viral clearance and sacrificed after several days (i.e. post "wash out"). This should demonstrate if renal toxicity is reversible or not.

3.B. SPECIFIC AIM 2: Defining Resistance to GS-5734 and Impact on Replication, Pathogenesis and Treatment.

Rationale. Reported biochemical studies with RSV suggest GS-5734 acts as a polymerase inhibitor through chain termination of nascent viral RNA[35]. CoVs are unique among RNA viruses as they encode a DE-D-Dh superfamily 3'-5' exoribonuclease (nsp14-ExoN) which exhibits proofreading activity during RNA synthesis. Thus, our demonstration that GS-5734 is active against WT (ExoN+) CoVs suggests a novel MOA that resists ExoN-mediated proofreading (**Fig. 2**), while many other related nucleoside inhibitors do not. Resistance selection with the nucleoside parent, GS-441524, and MHV yielded virus with mutations in two conserved residues in the CoV nsp12-RdRp (F476L/V553L) that when transferred to SARS-CoV via reverse genetics conferred resistance to GS-5734 **(Fig. 2**). In this aim, we address the following questions: **1)** Are mutational resistance patterns obtained during passage of virus in cell lines, primary human airway epithelial cells, and mice similar for MERS and SARS-CoV? **2)** Can resistance be improved through continued passage of resistant virus in the presence of GS-5734 and will additional resistance-enhancing mutations arise in nsp12-RdRp or other replicase proteins (i.e. nsp10, nsp14-ExoN, etc.)? **3)** Will resistance mutations effect virus replication, RNA synthesis, fidelity, and competitive fitness? **4)** Will resistance to GS-5734 increase or decrease susceptibility to other nucleoside analogs and mutagens? **5)** Will transfer of resistance mutations identified in MERS-CoV confer resistance in all CoV? **6)** Will resistance mutations effect virus replication, pathogenesis and treatment efficacy in mice infected with SARS-CoV or MERS-CoV? The proposed studies should provide insight into the MOA of GS-5734, define the limits and pathways to resistance, and establish whether resistance pathways are common across all CoV. Importantly, these data are key for IND licensure and are essential for the establishment of an informed clinical virology program. The (b)(6); (b)(3):7 U.S.C. § 8401 lab takes the key role in Aim 2, partnered with the Baric and Sheahan laboratories, while Gilead provides focus and prioritization.

3.B.1. Selection for GS-5734 Resistance In Vitro and In Vivo. In this subaim we will test whether passage of SARS-CoV and MERS-CoV in the presence of GS-5734 will result in resistance mutations obtained through passage of MHV (F476L/V553L) or new constellations of mutations, which may vary depending on the selection environment: Cell lines (Calu-3), primary human HAE cultures, and mice.

i. Passage for Resistance of MERS-CoV and SARS-CoV to GS-5734 in Calu-3 and HAE Cells. We adapt the approach that was successful for resistance generation with MHV (**Fig. 2, 9**) to SARS- and MERS-CoV using Calu-3 cells (VUMC) and HAE cultures (UNC). Calu-3 cells will be infected with SARS-CoV and MERS-CoV in the presence of GS-5734 at a concentration of 1-2 times the EC_{50}. Supernatant from cultures exhibiting visible cytopathic effect (CPE) will be blind passaged to naïve cells. With each passage drug concentration will increase stepwise. Supernatant and total RNA from each passage will be saved to monitor accumulation of resistance mutations and to have the option of restarting passage if increasing drug concentrations are too aggressive and virus is eradicated. For passage in HAE, cultures will be infected with SARS- or MERS-CoV at an MOI of 0.5 in presence of 1-2 times the predetermined EC_{50} value. At 4-day intervals, virus produced will be harvested via apical wash (half saved at -80 ˚C and half used for passage) and transferred to naïve cultures. Total RNA will be collected in Trizol for sequence analysis. With each passage, the concentration of GS-5734 will increase stepwise. We will perform 10-20 passages. Over time for both Calu-3 and HAE passage, the resistance phenotype of the population will be assessed and compared to parental virus in CPE-based antiviral assays in Vero cells. Resistance will be defined as at least a 5-fold shift in EC_{50}. In the event of virus extinction, passage will be restarted at lower concentrations and stepwise increases of drug will proceed at a slower rate. If the above protocols do not generate resistance, drug concentration and/or the time per passage can be modulated to produce more optimal selective pressure.

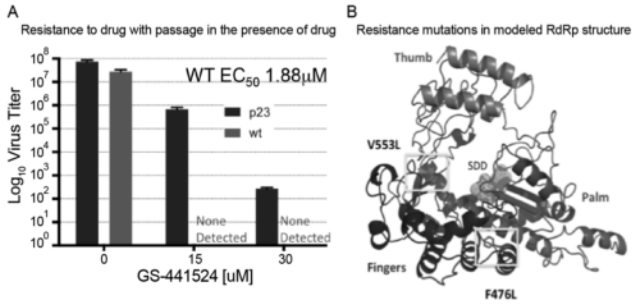

Figure 9. Resistant virus grows in the presence of drug and mutation locations in nsp12-RdRp A) Resistant "passage 23" MHV and WT virus production in the presence of GS-441524. **B)** Resistance mutations F476L and V553L in the nsp12-RdRp modeled structure. F476 and V553 residues are 100% conserved in coronaviruses.

ii. Sequence Analysis. The consensus population and minority variants will be defined across the full-length genome via next generation sequencing (NGS) of terminal-passage virus RNA from culture supernatant and

total RNA from the infected cell cultures[57]. Given the importance of nsp12, nsp10 and nsp14 on replication fidelity and sensitivity to RNA mutagens, non-synonymous (NS) mutations that arise in these genes will be given priority for reintroduction into parental virus via reverse genetics and confirmation of resistance. The modeling of mutations onto solved or predicted structures will provide insight into potential functional consequences of resistance mutations.

iii. Potential Outcomes and Alternatives. The selection for mutation of MHV nsp12 residues (F476L, V553L) that are 100% conserved in all known CoV and the transfer of resistance upon introduction of those mutations into SARS-CoV (**Fig. 2**) suggests a conserved MOA and favored pathway toward resistance for all CoVs, including SARS-CoV and MERS-CoV. However, given that only minor resistance is attained with F476L/V553L, it should be possible to obtain alternative or additional resistance mutations that provide more robust resistance. Additionally, because we are passaging in two completely different cellular environments (Calu-3 and HAE), the potential for obtaining multiple genetically distinct resistant virus lineages and identification of the limits of resistance will be maximized. Although unlikely, if we are unable to de novo select for resistance in MERS-CoV and SARS-CoV using Calu-3 or HAE cultures, we will initiate passage with partially resistant recombinant MERS-CoV and SARS-CoV encoding V553L and/or F476L.

3.B.2. In Vivo Selection for GS-5734 Resistance by SARS-CoV and MERS-CoV. To best model the complexity of resistance generation in humans, we utilize two complementary approaches, **acute passage** and **persistent infection**, to select for GS-5734 resistance in mice. These experiments will be performed initially with SARS-CoV as the animal models for acute infection and treatment (*Ces1c*$^{-/-}$) are available. Persistent infection will be performed after year 1 when *Ces1c*$^{-/-}$/*Rag1*$^{-/-}$ mice are available.

i. Acute Passage in *Ces1c*$^{-/-}$ Mice. Our preliminary in vivo studies with GS-5734 and SARS-CoV revealed that 50 mg/kg once daily resulted in complete protection from disease with significant decreases in virus titer while 10 mg/kg once daily afforded no protection, similar to vehicle treated controls. Using these data as a guide, we will treat groups of three 20 week-old mice with vehicle or GS-5734 (20 mg/kg) daily beginning the day prior to infection. Mice will be infected with 10^4 pfu mouse-adapted SARS-CoV MA15 and sacrificed 3 dpi for lung harvest. After homogenization and clarification, lung supernatants will be used to infect naïve mice. The dose of GS-5734 will be increased 5 mg/kg every fifth passage for 20 passages after which selective pressure will be increased via 25 mg/kg BID dosing for the final 5 passages. Virus from each passage will be titered by plaque assay to ensure consistent virus dosing during passage (10^4 pfu) and to maintain adequate population diversity for selection of resistant minority variants. Resistance mutations will be identified using NGS of total lung RNA. Resistant viruses arising from in vivo passage will be enriched in cell culture treated with 10 EC_{50} GS-5734, and then assessed for resistance in vitro. Putative resistance mutations will be engineered in the WT SARS-CoV background to confirm resistance phenotypes. Similar experiments will be performed with MERS-CoV in years 2-5 in Ces1c$^{-/-}$/288/330$^{+/+}$ mice.

ii. Persistent Infection in *Ces1c*$^{-/-}$/*Rag1*$^{-/-}$ Mice. We have shown that SARS-CoV can establish persistent high-titer replication in the lungs of adaptive immune deficient mice (*Rag1*$^{-/-}$) lasting over 60 days[32]. This system presents the unique opportunity to generate resistance mutants in relatively few animals through continuous dose escalation or intermittent dosing since constraints imposed by the adaptive immune response have been removed. We will generate double knockout *Ces1c*$^{-/-}$/*Rag1*$^{-/-}$ mice through mating of *Ces1c*$^{-/-}$ to *Rag1*$^{-/-}$ animals. 64 *Ces1c*$^{-/-}$/*Rag1*$^{-/-}$ mice will be infected with 10^5 pfu MA-SARS-CoV. After persistent infection is established and confirmed at 7 dpi, mice will be divided into two groups: *1) dose escalation and 2) pulse dosing*. For dose escalation, 32 mice will be administered 10 mg/kg GS-5734 (n = 16) or vehicle (n = 16) daily for one week, after which the dose will be increased at weekly intervals (20 mg/kg, 30 mg/kg, etc.) for a month. For pulse dosing (**Fig. 8**), 25 or 50 mg/kg GS-5734 or vehicle will be administered every other day to 16 animals per dose group (48 total) for a month. For both dose escalation and pulse dosing, four animals per group will be sacrificed each week to determine viral titer and track emergence of resistant variants via NGS. Similar experiments will be performed with MERS-CoV in years 2-5, using Ces1c$^{-/-}$/288/330$^{+/+}$/Rag1$^{-/-}$ mice.

iii. Potential Challenges and Alternatives. GOF approval will be requested prior to initiating these experiments, as we might unexpectedly select for increased pathogenic variants in parallel. Several factors could affect in vivo selection of resistance, such as drug metabolism and tissue- and organ-specific limits to drug penetration. If we cannot obtain resistance in vivo, we will initiate infections with SARS-CoV or MERS-CoV encoding minor resistance variant (F476L/V553L) nsp12 mutations facilitating selection of resistance at higher doses of drug and leading to the generation of new mutations that replace or augment those identified in vitro. Alternatively, we can use SARS-CoV ExoN(-) (mutator) virus for passage and selection with lower dose

ranges of GS-5734. Although this virus is attenuated in vivo and more sensitive to GS-5734, it can establish persistent infection in Rag1$^{-/-}$ mice. With a mutation rate >20X that of WT virus, ExoN(-) virus should increase the rate at which resistance mutations are generated. In addition, other resistance pathways may emerge in the absence of ExoN-mediated fidelity.

3.B.3. Impact of Resistance Mutations on Virus Replication and Fitness In Vitro. Prior to widespread use of GS-5734 in humans, it is critical to understand if resistance mutations will effect virus replication and fitness in vitro and in vivo. Our preliminary studies demonstrate that nsp12-RdRP resistance mutations (F476L, V553L) confer no superiority to WT virus in single-round competition. However, the overall fitness cost and impact on replication for MERS-CoV and SARS-CoV remains unknown. The nsp12 F476L and V553L mutations and any new resistance mutations arising during MERS-CoV and SARS-CoV passage will be engineered alone and together in both biologically occurring and combinatorial sets in the SARS-CoV and MERS-CoV isogenic backgrounds, with and without an encoded nano-Luciferase (nLUC) reporter.

i. Resistance, Replication and RNA Synthesis. The degree of GS-5734 resistance will be determined in standard antiviral dose response assays in Calu-3 cells (i.e. 10-point dose response) using nLUC expressing reporter viruses as we have in **Fig. 2**. Newly discovered resistant variants will be compared to WT and F476L/V553L mutant viruses. Infectious virus production will also be compared in the absence of drug in single cycle (high MOI) and multi-cycle (low MOI) infection assays in Calu-3 cells. Genomic and subgenomic RNA levels will be quantified by RT-qPCR at times corresponding to peak viral RNA synthesis (24-48 hpi).

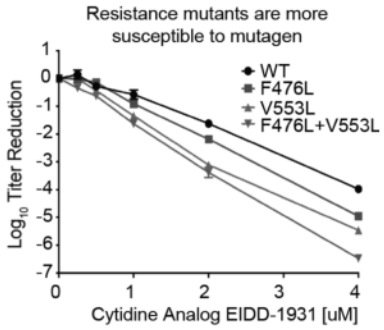

Figure 10. GS-5734 resistance mutations increase sensitivity to cytidine analog EIDD-1931.

ii. Cross-Sensitivity of GS-5734 Resistance Mutants. We have exciting preliminary evidence indicating that GS-5734 (adenosine analog) resistance mutations F476/V553L increase sensitivity to a structurally distinct cytidine analog (EIDD-1931) (**Fig. 10**). As a basis for understanding MOA, the potential for future combination treatment, and the limitations of resistance emergence, we will determine if GS-5734 resistance mutations alter sensitivity to other nucleoside analogs or mutagens. WT and recombinant mutant SARS-CoV and MERS-CoV will be compared in standard antiviral dose response assays in Calu-3 cells using nLUC expressing reporter viruses. Potential test molecules include mutagens 5-fluorouracil (5-FU), ribavirin (RBV) and 5-azacytidine (5-AC) and chain terminators and polymerase inhibitors 2' C-methyl adenosine (2'CMA) and EIDD-1931. Cross-resistance between GS-5734 and all classes of nucleosides would suggest a general fidelity increase, whereas decreased sensitivity to one or more compounds would be consistent with nucleotide selectivity as the mechanism of GS-5734 resistance and support combination therapy for treatment and prevention of resistance emergence.

iii. Competitive Fitness. Viruses encoding the nsp12-RdRp F476L/V553L mutations have a small plaque phenotype yet appear to replicate to WT levels with no apparent fitness cost. Coupled with the fact that resistance was very difficult to select, these results underscore the importance of determining the competitive fitness of selected resistance mutations. For in vitro analysis, MERS-CoV and then SARS-CoV resistant mutants will be competed against WT virus by co-infection of Vero cells (in absence of GS-5734) and Calu-3 cells (in absence or presence of GS-5734) at ratios of 1:1, 9:1, and 1:9, followed by five sequential passages of culture supernatants. We will quantify WT and mutant virus intracellular and virion (supernatant) genomic RNA by RT-qPCR[32]. This approach reveals subtle changes in competitive fitness that might not manifest in single-round infection studies. Mutant viruses with replicative fitness equal to or increased relative to WT virus will be tested in HAE cells and prioritized for pathogenesis and GS-5734 protection studies in vivo

3.B.4. Effect of Resistance Mutations on In Vivo Treatment and Pathogenesis. The goal is to determine if resistance mutations alter in vivo pathogenesis and/or confer resistance upon treatment with GS-5734 in vivo. To identify high priority candidates for detailed studies, 20-week-old *Ces1c*$^{-/-}$/288/330$^{+/+}$ will be administered 25mg/kg GS-5734 (n=6/virus) or vehicle (n=6/virus) subcutaneously twice daily (BID) beginning 1 day prior to infection. Mice will be infected with 1 LD_{50} of parental (i.e. MERS-15 nLUC) or isogenic resistant mutant virus expressing nLUC. Weight loss, virus replication via IVIS Lumina III, pulmonary function (whole body plethysmography, WBP) and morbidity will be evaluated daily through day 6 post-infection. These studies will reveal if resistance mutations affect pathogenesis and the degree to which mutations confer resistance to treatment. For select mutants demonstrating attenuated or increased replication/disease in the presence/absence of drug, detailed studies will be performed. Briefly, 20-28 week old mice will be infected with

10^4, 10^5 or 10^6 pfu WT parental or isogenic resistance mutant virus (n = 30 per virus; 10 mice/dose). The virus dose range correlates to a spectrum of MERS-15 and SARS-CoV MA15 pathogenesis spanning mild weight loss (10^4), significant weight loss and intermediate survival (10^5), and significant weight loss with death (10^6)[76]. Pulmonary function will be measured daily by WBP. On 3 and 6 dpi, five mice will be sacrificed and lungs harvested to measure viral load, immunohistochemistry, pathology, and inflammatory cytokines by BioPlex. Also at this time, complete blood count will be determined via Vetscan HM5c. Our preliminary data suggest that SARS-CoV-MA15 F475L/V553L is not attenuated in Balb/c mice at the high dose (**Fig. 7**).

3.B.5. Expected Outcomes and Alternative Approaches. There are scant data on fitness alterations stemming from CoV polymerase mutations, with the exception of studies in our group showing that mutations at V553 in nsp12 increase fidelity and decrease competitive fitness in vitro[77]. We do know that mutations in the proofreading nsp14-ExoN are attenuating in animals, and that selection for F476L/V553L GS-5734 resistance mutations were difficult to achieve[32]. Overall, we expect the pathogenesis of resistant mutants to be similar to or attenuated relative to parental virus, most likely detected by changing LD_{50}. However, fitness in vitro may not correlate with in vivo fitness, and pathogenesis may not correlate directly with altered fitness. Thus, the functional consequence of resistance mutations must be evaluated in vivo in a complex organism that is susceptible to disease. Given that EC_{50} values for F475L/V553L-containing viruses are five-fold greater than WT virus, we expect resistance mutants to cause disease with GS-5734 treatment that is protective for WT viruses (25mg/kg BID). However, if resistance mutants cannot evade treatment and do not cause disease, GS-5734 dose and/or frequency will be diminished until a regimen is found that promotes resistant virus disease and prevents WT virus disease. Integrated knowledge of resistant virus fitness and pathogenic potential will inform the clinical virology program as GS-5734 progresses toward human trial.

3.B.6. Future Directions – Biochemistry, Molecular Mechanism and Evasion of Proofreading. The above studies will yield deep insights into the target of GS-5734 and crucial structure-function relationships in nsp12-RdRp, nsp14-ExoN, and other replicase proteins. We are currently working to establish in vitro polymerase assays for both model CoV (MHV) and SARS-CoV. Toward this end, we have purified nsp10, nsp12, and nsp14 and expect to be able to generate a model for directly testing GS-5734 molecular MOA using isolated nsp12-RdRp and possibly the multicomponent replicase complex containing nsp12, nsp13, nsp14, and nsp10. This is a separate project, but mutations associated with resistance will guide biochemical studies of RdRp function and illuminate the molecular basis of GS-5734 escape from nsp14-ExoN proofreading activity.

3.C SPECIFIC AIM 3: Defining the Mechanism of Action of GS-5734.

Rationale. Studies with RSV suggest that GS-5734 exerts its antiviral effect on the RdRp through chain termination of nascent viral RNA but the MOA for CoV remains unknown. Aside from the direct effects on virus replication, GS-5734 treatment may also cause the generation of incomplete, partial, or mutated viral RNA, whose recognition by innate sensors enhance antiviral innate immune responses resulting in loss of viral fitness or attenuated viral pathogenesis. Using NGS, Sanger sequencing and RNA FISH, we will determine the effect of GS-5734 treatment on viral positive and negative sense RNA synthesis while simultaneously examining the host transcriptional response to infection in vitro and in vivo, thus uniting the observed antiviral effect of GS-5734 with effects on viral transcription and replication. While Aim 2 is focused on identifying the viral gene(s) targeted by GS-5734 through the selection of resistance mutants, Aim 3 provides independent complementary data by measuring the downstream effects of treatment on replication. We expect that comprehensive data provided by Aims 2 and 3 will demonstrate a MOA that involves direct targeting of virus. Confirming the “on target” antiviral effect while interrogating the potential for “off target” effects will inform future human clinical trials by providing insight into potential adverse side effects. Dr. Sheahan, partnered with the Baric and [redacted] laboratories, will lead these efforts in close consultation and support by our Gilead partners.

(b)(6); (b)(3):7 U.S.C. § 8401

3.C.1. The Effect of GS-5734 on CoV RNA Transcription In Vitro.

Hypothesis: GS-5734 treatment directly affects SARS- and MERS-CoV viral RNA synthesis, resulting in mutated and/or truncated RNA species that are more readily detected by host innate immune sensors. **Rationale:** Our preliminary data shown in **Fig. 2** suggests that the viral RdRp is targeted by GS-5734, but the downstream effects on virus replication remain unknown. In this subaim, we characterize the effects of GS-5734 treatment on viral RNA species in primary human HAE cultures, which contain cells targeted by both SARS- and MERS-CoV in vivo and are competent in their ability to induce innate immunity. While cell lines (e.g., Vero cells) used to create virus stocks are less expensive and genetically homogenous as compared to HAE from various human donors, they are dysfunctional in multiple aspects of their cell biology, including cell cycle regulation and the induction of innate immunity. If the generation of altered or truncated viral transcripts

with treatment leads to enhanced recognition of viral pathogen-associated molecular patterns (PAMPs), these studies must be performed in cells with intact innate immune sensing and effector networks, such as HAE.

i. Experimental Design: From three different human donors, we will infect HAE cultures with SARS- or MERS-CoV in the presence of vehicle or a range of GS-5734 known to be strongly (1 μM), moderately (0.1 μM), or not antiviral (0.01 μM). Two distinct RNA populations will be isolated from each culture. First, to determine the effects of treatment on viral genome replication fidelity, genomic RNA from viral particles will be isolated from apical washes of HAE cultures. Second, we will isolate total cellular RNA from HAE in Trizol to explore the effect of treatment on genomic and subgenomic viral RNAs and host transcription. Strand-specific libraries will be constructed according to manufacturer's protocols (Illumina TruSeq Stranded RNA Library Preparation Kit), and RNA-seq data will be analyzed using CLC Genomics Workbench.

ii. Expected Results, Potential Pitfalls and Alternative Approaches. Since host genetics can have a profound effect on the host response and outcome of virus infection, we will use of HAE derived from three different human donors to account for potential donor effects. We have much experience using deep sequencing to analyze CoV RNA species[57]. If GS-5734 is incorporated into viral RNA, it will appear as an adenine. Thus, upon analysis, if genomic (apical washes and total RNA) and subgenomic viral RNA (total RNA) from drug-treated cultures contain significant increases in adenine mismatches as compared to DMSO-treated cultures, this would suggest that GS-5734 antiviral effect is mediated by loss of replicative fidelity and error catastrophe. Alternatively, we may find an abundance of very short viral RNAs in treated cultures as compared to DMSO, which would be suggestive of chain termination. We expect to see significantly elevated levels of interferon-stimulated gene transcripts if drug treatment leads to generation of altered or increased abundance of viral RNA PAMPs. Alternatively, drug treatment may inhibit expression of virally encoded interferon antagonists, leading to more effective innate immune responses, ISG expression and increased inhibition of virus replication. It is also possible that the antiviral effect of GS-5734 is predominantly due to direct reduction in virus replication. Since HAE only contain approximately $1x10^6$ cells per culture, we may pool biological replicates to have enough input RNA for these studies. Alternatively, we will perform similar studies in Calu-3 cells, a continuous airway cell line that supports both SARS- and MERS-CoV infection[44].

3.C.2. The Effect of GS-5734 on CoV RNA Transcription In Vivo.

Hypothesis: GS-5734 treatment directly affects SARS- and MERS-CoV viral RNA synthesis, resulting in mutated and/or truncated RNA species that are more readily detected by host innate immune sensors. **Rationale:** In this subaim, we extend the mechanistic in vitro studies in Aim 3.C.1 to our in vivo efficacy models for both MERS-CoV and SARS-CoV. Thereby, we will define GS-5734 MOA in tissues containing the diverse array of cells targeted by virus as well as the leukocyte populations that likely play a role in the response to infection (e.g., alveolar macrophages and dendritic cells). Our preliminary data demonstrate that SARS-CoV tropism is different in vehicle-and GS-5734-treated animals, but the mechanism remains unknown. Our goal is to model the complex interplay between the variety of infected cells and non-infected but affected cells, which can only be studied within in vivo models of disease.

i. Experimental Design: GS-5734 (25 mg/kg) (n = 16) or vehicle (n = 16) will be administered subcutaneously to 20-week-old *Ces1c*$^{-/-}$, *Ces1c*$^{-/-}$/STAT1$^{-/-}$ or *Ces1c*$^{-/-}$/Rag1$^{-/-}$ mice beginning day -1 and given twice daily throughout the experiment. STAT1$^{-/-}$ mice cannot clear SARS-CoV infection and 100% mortality occurs by day 10, allowing us to assess whether GS-5734 can clear in the absence of STAT1 regulated innate immune or adaptive immune responses (Rag1$^{-/-}$)[78, 79]. Mice will be intranasally infected with 10^4 pfu of SARS-CoV MA15. On days 1, 2, 3 and 4 post-infection, 4 mice will be sacrificed and lungs will be harvested for virus titer, pathology and total RNA, which will be isolated and processed for sequencing according to methods described above. Once the MERS-CoV mouse model (*Ces1c*$^{-/-}$/288/330$^{+/+}$) has been established (Aim 3.A.3), similar studies could be performed with MERS-CoV.

ii. Expected Results and Potential Pitfalls. Given the increased cellular complexity of the in vivo pulmonary environment as compared to monocultures or even HAE cultures, we expect the host transcriptional response to be different in complexity, identity and kinetics as compared to our in vitro data. We have much experience assessing the host response to SARS-CoV in mice and do not anticipate technical difficulties with these experiments[44, 71, 76]. In WT mice, we do expect a greater induction of innate immune gene transcription with treatment as compared to vehicle treated animals. If true, NGS sequencing of viral RNAs should reveal if a more potent host response is due to the creation of altered viral RNA PAMPs or defective genomes. Alternatively, drug treatment also might inhibit expression of virally encoded interferon antagonists, leading to more effective innate immune responses and increased inhibition of virus replication. It is also possible that

drug treatment will not alter induction of innate immune responses in vivo and that the antiviral effect of GS-5734 is predominantly due to direct reduction in replication. If this is the case, then GS-5734 should clear infection in both the RAG1$^{-/-}$ or STAT1$^{-/-}$ mouse models. Alternatively, innate immunity or adaptive immunity or both may be essential for GS-5734 mediated clearance, critical information when considering patient care in immunosenescent or immune deficient humans. NGS data on viral RNAs will be mined as done above to determine if treatment correlates with an over-abundance of mutated or truncated viral transcripts. Due to the increased variety of cells targeted by both SARS- and MERS-CoV present in vivo, the observed effect on viral RNA species may be an amalgamation of multiple predicted signatures (i.e. mutated transcripts, chain termination, truncated transcripts, etc.).

3.C.3. Visualizing the Antiviral Effect at the Single Cell Level via RNA FISH.

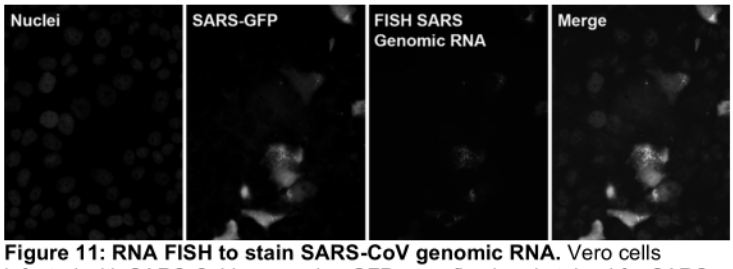

Figure 11: RNA FISH to stain SARS-CoV genomic RNA. Vero cells infected with SARS-CoV expressing GFP were fixed and stained for SARS-CoV genomic RNA using 48 different Quasar 570 probes targeting ORF1a.

Hypothesis: GS-5734 treatment directly affects SARS- and MERS-CoV viral RNA synthesis, resulting in mutated and/or truncated RNA species that are more readily detected by host innate immune sensors. **Rationale:** Unlike the total RNA "population" approaches taken in subaims 3.C.1 and 3.C.2., dimensional architecture is preserved in single-molecule RNA fluorescence in situ hybridization (FISH), allowing for the collection of multiplex data at the single-cell level. Using RNA FISH, we have successfully visualized SARS-CoV genomic RNA (**Fig. 11**). We will build upon these preliminary data by co-staining for either negative sense CoV RNA or RNA encoding classical interferon stimulated genes (i.e. IFN-β and IFIT1). We will perform these experiments in MERS-and SARS-CoV-infected Calu3 cells as we have detailed transcriptomic and proteomic data to guide the experimental design[44].

i. Experimental Design: Calu-3 2B4 cells grown on glass coverslips will be infected at a low MOI with SARS- or MERS-CoV and treated concurrently with vehicle or a range of GS-5734 concentrations known to be very strongly (10 μM), strongly (1 μM), moderately (0.1 μM), or not (0.01 μM) antiviral. 12-24 hpi, coverslips will be fixed and stained for RNA FISH. The 48 different fluorescent probes per target RNA are resolved as a single spot via microscopy. Using a Nikon wide-field microscope, images of random cells will be taken for each condition, and RNA spots will be quantified using free software developed by Dr. Arun Raj at the University of Pennsylvania. Various probe and fluorophore combinations will be used to answer different biological questions. To determine if GS-5734 prevents the origination of negative strand viral RNA, we will use probes against positive- (Quasar 570 nm) and negative-sense (Quasar 670) viral RNA. To determine if virus-infected and drug-treated cells are more efficient at inducing innate immunity, we will use probes against viral positive-sense RNA (Quasar 570 nm) and either IFN-β, IFIT1 or other ISGs (Quasar 670) mRNAs.

ii. Expected Results, Potential Pitfalls and Alternative Approaches. We have successfully used single-molecule FISH for multiplexed detection of both HCV RNA and host innate immune transcripts[80]. Thus, we do not anticipate technical problems. The wide range of drug concentrations should yield a full spectrum of biological phenotypes for imaging, from rampant replication (0.01 μM and DMSO vehicle) to full abrogation of replication (10 μM). We will initially treat concurrent with infection, but will also explore treatment hours before infection to pre-load cells with active TP and hours after infection to allow for the establishment of replication complexes. To maximize clinical application, we will perform similar studies in HAE cultures once we have determined optimal conditions for RNA FISH. This technique can also be adapted to mouse lung sections to visualize the kinetic impact of treatment in the lung (e.g., conducting airways, alveoli, etc.).

3D. A Timeline for the comprehensive preclinical evaluation of GS-5734 for MERS-CoV.

Colored boxes per quarter per year indicate the duration of work for each subaim. White boxes indicate absence of work.

		Year 1				Year 2				Year 3				Year 4				Year 5			
Aim	Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1.1	Isolation of recombinant zoonotic 2D viruses																				
1.2	Antiviral activity, toxicity and metabolism in primary human lung and immune cells																				
1.3	Prophylactic and therapeutic treatment in *Ces1c*$^{-/-}$/288/330$^{+/+}$ mice with MERS-CoV																				
1.4	In vivo PK Analysis inGS-5734 in *Ces1c*$^{-/-}$/288/330$^{+/+}$ mice																				
1.5	In vivo efficacy in NHP models of MERS- and SARS-CoV																				
2.1a	In vitro selection for resistance in Calu and HAE cell cultures																				
2.1b	In vivo selection for SARS- and MERS-CoV resistance																				
2.1c/d	Impact of resistance mutations on virus replication and fitness in vitro and in vivo																				
3.1	Impact of GS-5734 on transcription in vitro																				
3.2	Impact of GS-5734 on transcription in vivo																				
3.3	Visualizing the antiviral effect via single molecule RNA FISH																				

5. Protection of Human Subjects

Risks to the Subjects.

a. Human Subjects Involvement and Characteristics: Specimens are obtained from human subjects and are handled per protocols approved by the UNC Institutional Committee on the Protection of the Rights of Human Subjects (IRB). The samples are derived from excess surgical pathology materials and anonymous or identifiable cadaveric organ donors. Our studies require some essential microbiology and genetic data to be extracted from the patient record and some cadaveric specimens are provided with personal identifying data (PID). Thus, procedures are in place to ensure patient confidentiality as described below. An annually renewed IRB "umbrella" protocol (#03-1396) has been in effect for other prior and current studies. A new protocol in the name of the current R01 will be obtained under NIH JIT regulations. Excess surgical pathology tissues are obtained from patients undergoing lung transplantation, lobectomy or pneumonectomy. Organ donor lungs not suitable for transplantation but still useful for cell harvest are obtained through locally through Carolina Donor Services, and nationally through the National Disease Research Interchange (Philadelphia, PA) or the International Institute for the Advancement of Medicine (Edison, NJ). Age, sex, and ethnic background will not be considered when obtaining specimens, and are expected to reflect those of the U.S. population of patients with CF and general organ donors. Individuals with known infection or evidence of infection with human immunodeficiency virus, hepatitis B, hepatitis C, syphilis, or tuberculosis will be excluded to protect the safety of research personnel. However, all potentially biohazardous samples are handled using standard precautions as specified in our Laboratory Safety Plan. Twelve lung specimens, as indicated in the Enrollment Plan, will be procured to meet Project needs.

b. Sources of Materials: Research specimens, consisting of excess surgical pathology tissue, are obtained from the University of North Carolina Hospitals and Duke University Lung Transplantation programs and Departments of Pathology. Sub transplant quality lungs, useful for research are obtained from Carolina Donor Services and other non-profit organizations that provide biomaterials. Patient demographic data (age, gender, clinical diagnosis, and pertinent pulmonary function and microbiology data) are extracted from the medical records and/or reports of 3rd party providers and are stored in confidential files.

c. Potential Risks: No risks beyond those associated with the elective surgical procedures or organ donation are imposed by these studies. Donor confidentiality is protected.

Adequacy of Protection Against Risks

a. Recruitment and Informed Consent: Patients are recruited and consented using IRB-approved forms by referring physicians listed on the protocol and/or the Core Director. Uniform direct consent is not practicable or feasible because some donors are deceased (i.e., cadaveric organ donors). For these specimens, authorized representatives provide consent for research use of tissues.

b. Protections Against Risk: Subject confidentiality is maintained by assigning an anonymous code to each tissue. Ultimate users of the cells and tissues do not receive any personal identifying data (PID). Hard copies of the consent forms are storied in locked files. Data summarizing the underlying diagnosis, gender, age and other information is maintained in secure, password protected computer files.

Potential Benefit of the Research to the Subjects and Others: At this point, there are no direct benefits to the patients. However, by defining epithelial physiologic and biologic processes relevant to the development and treatment of disease, these studies are of value to individuals with respiratory tract abnormalities and society in general.

Importance of the Knowledge to be Gained: These studies may ultimately lead to novel therapeutic approaches for viral infection and other lung diseases.

6. Data and Safety Monitoring Plan: Not a clinical trial, not applicable.

Clinical Trials.Gov Requirements: Not a clinical trial, not applicable.

Exemption Status: The use of excess surgical pathology materials and anonymous cadaveric organ donor tissue are often considered to be exempt from IRB review. However, our studies require some essential microbiology and genetic data to be extracted from the patient record, with procedures in place to ensure patient confidentiality. An annually renewed IRB "umbrella" protocol (#03-1396) has been in effect for other prior and current studies. A new protocol in the name of the current R01 will be obtained under NIH JIT regulations.

Inclusion of Women and Minorities: The studies outlined in this proposal use excess excised human surgical pathology tissues and tissues from organ donors that were deemed unsuitable for transplant but useful for research. These tissues are representative of the populations undergoing the relevant surgical procedures in our geographical area or all organ donors within the procurement pool of the supplying agencies, including minorities. Cystic fibrosis is predominantly, but not exclusively, a disease of Caucasians, which will be reflected in the population. The Core accepts human samples regardless of gender or race.

Inclusion of Children: The studies outlined in this proposal use excess excised human surgical pathology tissues and tissues from organ donors that were deemed unsuitable for transplant but useful for research. These tissues are representative of the populations undergoing the relevant surgical procedures in our geographical area or all organ donors within the procurement pool of the supplying agencies and may include children. Lung transplantation occurs predominantly, but not exclusively, in adults, which will be reflected in the population. The Core accepts human samples regardless of age.

9. Vertebrate Animals.

The goal of these studies is to accelerate the preclinical development of lead broad-spectrum antiviral GS-5734 to treat MERS-CoV infections. Animal research plays a key role in the development of medicine by providing evidence of therapeutic efficacy and insight into the pharmacokinetics, metabolism, safety and potential adverse events. This information is essential for the progression to human clinical trial. All rodent animal experiments will be performed at the University of North Carolina in dedicated facilities under the direction of the research PI. Prior to infection studies, the animals will be maintained in Sealsafe^{TM} HEPA-filtered air in/out unit or compatible system for at least one week prior to virus challenge. In addition, our laboratory personnel inspect animals daily and any animal in distress is immediately euthanized (moribund, unresponsive, loss of more than approved percentage of starting weight). Animal care and housing at UNC follows IACUC recommendations and all personnel have attended mandatory IACUC training courses. A trained veterinarian is always on call to assist with problems in animal care and husbandry. We will utilize mice (UNC) as well as non-human primates (UTMB). Below we summarize the description of procedures for each specific Aim, justifications, minimization and pain and distress and methods for euthanasia.

1. Procedures. All rodent work will be done at UNC-Chapel Hill in accordance with the Guide for the Care and Use of Laboratory Animals. The minimum numbers of animals will be used in order to achieve our experimental goals with statistical significance. We aim to monitor virus replication in live animals over time using in vivo imaging rather than traditional means of sacrificing multiple cohorts to gain similar data. In vivo imaging studies require fewer animals in step with the 3R principle (replacement, reduction and refinement).

Specific Aim 1. Refining the Pharmacokinetics and Pharmacodynamics of GS-5734. We have done extensive work with demonstrating efficacy of GS-5734 against SARS-CoV but have not performed studies with MERS-CoV due to model availability. We generate a transgenic mouse to facilitate drug testing against MERS-CoV.

A. Prophylactic efficacy of GS-5734 in 20 week old female Ces1c$^{-/-}$/288/330$^{+/+}$ with MERS-CoV.

a. Vehicle (n = 6) and GS-5734 treated (n = 6) and intranasally infected with MERS-15 nLUC.
   i. Vehicle or GS-5734 is delivered via subcutaneous injection twice daily.
   ii. Mice will be monitored for weight loss, virus replication will be assessed by IVIS Lumina III and pulmonary function by whole body plethysmography every day for 6 days after which animals will be sacrificed by isofluorane overdose.
   iii. IVIS Lumina III requires isofluorane anesthesia.
   iv. Total of three experiments for statistical significance= 36 mice

b. Vehicle (n = 12) and GS-5734 treated (n = 12) and intranasally infected with MERS-15 nLUC.
   i. Vehicle or GS-5734 is delivered via subcutaneous injection twice daily.
   ii. Mice will be monitored for weight loss and pulmonary function by whole body plethysmography every day.
   iii. On days 3 and 6 post infection, half of each cohort will be sacrificed by isofluorane overdose. Lungs (virus titer, pathology, antigen staining) and blood (cytokine analysis, complete blood count) will be harvested for analysis.
   iv. Total of three experiments for statistical significance= 72 mice

B. Therapeutic efficacy of GS-5734 in 20 week old female Ces1c$^{-/-}$/288/330$^{+/+}$ with MERS-CoV.

a. Vehicle (n = 6), GS-5734 treatment beginning day -1 (n = 6), GS-5734 treatment beginning day +1 (n = 6), GS-5734 treatment beginning day +2 (n = 6) and intranasally infected with MERS-15 nLUC.
   i. Vehicle or GS-5734 is delivered via subcutaneous injection twice daily.
   ii. Mice will be monitored for weight loss, virus replication will be assessed by IVIS Lumina III and pulmonary function by whole body plethysmography every day for 6 days after which animals will be sacrificed by isofluorane overdose.
   iii. IVIS Lumina III requires isofluorane anesthesia.
   iv. Total of three experiments for statistical significance= 72 mice

b. Vehicle (n = 12), GS-5734 treatment beginning day -1 (n = 12), GS-5734 treatment beginning day +1 (n = 12), GS-5734 treatment beginning day +2 (n = 12) and intranasally infected with MERS-15 nLUC.
   i. Vehicle or GS-5734 is delivered via subcutaneous injection twice daily.
   ii. Mice will be monitored for weight loss and pulmonary function by whole body plethysmography every day.

iii. On days 3 and 6 post infection, half of each cohort will be sacrificed by isofluorane overdose. Lungs (virus titer, pathology, antigen staining) and blood (cytokine analysis, complete blood count) will be harvested for analysis.
iv. Total of three experiments for statistical significance= 144 mice

C. Prophylactic efficacy of GS-5734 in 12-18 month old female Ces1c$^{-/-}$/288/330$^{+/+}$ with MERS-CoV or SARS-CoV.

a. Numbers, metrics and endpoints will be the same as those in Specific Aim 1.A.a
    i. Total of three experiments for statistical significance= 36 mice/virus = 72 total.
b. Numbers, metrics and endpoints will be the same as those in Specific Aim 1.A.b.
    i. Total of three experiments for statistical significance= 72/virus = 144 total.

D. Therapeutic efficacy of GS-5734 in 12-18 month old female Ces1c$^{-/-}$/288/330$^{+/+}$ with MERS-CoV or SARS-CoV.

a. Numbers, metrics and endpoints will be the same as those in Specific Aim 1B.a.
    i. Total of three experiments for statistical significance= 72 mice/virus = 144 total.
b. Numbers, metrics and endpoints will be the same as those in Specific Aim 1B.b.
    i. Total of three experiments for statistical significance= 144 mice/virus = 288 total.

E. **Pharmacokinetic studies to be performed by Gilead Sciences and funded through a different mechanism**

F. Non-human primate models to assess prophylactic (SARS-CoV) and therapeutic efficacy (MERS- and SARS-CoV). These studies will be performed at UTMB/GNL.

a. All NHPs will be ordered from UTMB-approved animal vendors. Animals will be treated with a lead antiviral agent through a subQ route (one dose [10 mg/Kg]/per day), starting at 8 hrs before (preventive) or after (treatment) challenge with 7.5 $TCID_{50}$ of SARS-CoV or MERS-CoV, via a combination of intranasal (i.n.), intratracheal (i.t.), and oral and ocular (o/o) routes, to test the efficacy of the lead antiviral agent. NHPs will be anesthetized by the GNL veterinary staff before any manipulation. Animals will be monitored daily for their complete blood count with differential, blood chemistry, temperature, CT scanned for pneumonia, and morbidity (if any). Animals will then be euthanized at the end of the study (likely 72 hrs after infection) and the relevant tissues will be collected for virology and histopathology analysis.
    i. **Species/strain**: African Green Monkey (*Chlorocebus aethiops*) and rhesus macaques (*Macaca mulata*)
    ii. **Age**: 3-6 years old
    iii. **Sex**: Male and Female
    iv. **Numbers**: 36 total (N=18 each species)

Specific Aim 2. Defining GS-5734 Resistance to GS-5734 and Impact on Replication, Pathogenesis and Treatment. The goals of these studies are to generate drug resistance in vivo and assess the effect of drug resistance on viral pathogenesis. These studies are key to the establishment of an informed clinical virology program prior to human clinical trial.

G. **Acute passage of SARS-CoV MA15 or MERS-15 in 20 week old female Ces1c$^{-/-}$/288/330$^{+/+}$**

a. The goal is to passage virus in vivo in the presence of increasing doses of virus to select for viruses resistant to drug.
b. Vehicle (n = 3) and GS-5734 treated (20mg/kg) (n = 3) intranasally infected with SARS-CoV MA15 or MERS-CoV.
    i. Vehicle or GS-5734 is delivered via subcutaneous injection once daily.
    ii. 3 days post infection, mice will be sacrificed by isofluorane overdose, lungs will be homogenized and clarified supernatants will be used to intranasally infect naïve mice treated similarly as those above in “a”. This process would complete one passage.
    iii. Dose of GS-5734 will increase 5mg/kg ever five passages and we intend to perform 20 passages.
    iv. Total number of mice for passage 180 mice/virus = 360 total.

H. **Persistent infection with SARS-CoV MA15 or MERS-15 in 20 week old female Ces1c$^{-/-}$/288/330$^{+/+}$**

a. The goal is to persistently infect immunodeficient mice and deliver drug in escalating doses or pulses in order to select for drug resistant virus.
b. 68 mice will be infected with SARS-CoV MA15 or MERS-CoV and at 7 dpi, mice will be divided into two groups.
    i. Dose escalation. 16 mice will be administered vehicle and 16 mice will receive 10mg/kg GS-5734 subcutaneously once daily for one week after which the dose will increase every week for a month (10mg/kg, 20mg/kg, 30mg/kg, etc). Vehicle groups only ever receive vehicle. Mice will then be sacrificed by isofluorane overdose and the lungs will be harvested to sequence resistant virus and isolate resistant viral variants.
    ii. Pulse dosing. Mice will be administered vehicle (n = 12), 25mg/kg GS-5734 (n = 12) or 50mg/kg (n = 12) ever other day for a month. Mice will then be sacrificed by isofluorane overdose and the lungs will be harvested to sequence resistant virus and isolate resistant viral variants.
    iii. If not necessary to repeat, 68 mice/virus for a total of 136. If necessary to repeat, 136/virus for a total of 272.

I. **Effect of resistance mutations with MERS-CoV or SARS-CoV MA15 on in vivo treatment and pathogenesis in Ces1c$^{-/-}$/288/330$^{+/+}$**
    a. Vehicle (n = 6/virus) and GS-5734 treated (n = 6/virus) and intranasally infected with MERS-15 nLUC or MERS-15 nLUC Resistant mutant virus. For SARS-CoV, infection will be with SARS-CoV MA15 nLUC or SARS-CoV MA15 nLUC Resistant mutant virus.
        i. Vehicle or GS-5734 is delivered via subcutaneous injection twice daily.
        ii. Mice will be monitored for weight loss, virus replication will be assessed by IVIS Lumina III and pulmonary function by whole body plethysmography every day for 6 days after which animals will be sacrificed by isofluorane overdose.
        iii. This will be repeated approximately 3 times/virus background (i.e. SARS-CoV or MERS-CoV) = 144

J. Comprehensive evaluation of SARS- or MERS-CoV resistant mutant pathogenic potential.
    a. 20-28 week old female Ces1c$^{-/-}$/288/330$^{+/+}$ mice will be infected with 10^4, 10^5 or 10^6 pfu of WT or resistant virus. 10 mice per dose per virus = 30 mice/virus.
        i. On 3 and 6 dpi, five mice per condition will be sacrificed and lungs harvested to measure viral titers, pathology, and complete blood count.
        ii. Total of three experiments for statistical significance= 180 mice for SARS-CoV and 180 mice for MERS-CoV.

Specific Aim 3. Defining the MOA of GS-5734. The goals of these studies determine the effect of drug on virus transcription and solidify the mechanism of action.

K. 20-week old Ces1c$^{-/-}$, Ces1c$^{-/-}$/STAT1$^{-/-}$ or Ces1c$^{-/-}$/Rag1$^{-/-}$ will be administered GS-5734 (25 mg/kg) (n = 16/strain) or vehicle (n = 16/strain) subcutaneously beginning day -1 and given twice daily throughout the experiment. Mice will be intranasally infected with 10^4 pfu of SARS-CoV MA15. On days 1, 2, 3 and 4 post-infection, 4 mice will be sacrificed and lungs will be harvested for virus titer, pathology and total RNA.
    a. Per mouse strain = 32 mice for 96 mice per experiment. Total of three experiments for statistical significance = 288 total.

2. Justifications

This proposal aims to accelerate the preclinical development of GS-5734 in preparation for filing an Investigational New Drug (IND) with the FDA. The work described above will provide key proof of principle data demonstrating abrogation of MERS-CoV disease in mice. We also aim to define the mechanism of action, identify the viral genetic pathways that lead to resistance and determine if resistance effects viral pathogenesis. These studies cannot be done without vertebrate animals. There is no *in vitro* system that accurately mimics virulence of either CoV that is seen in animals or humans and would predict the outcome of infection. While critical viral/host cell interactions can be studied in cell culture, there is no substitute for measuring immunologic and pathologic processes in the intact animal to elucidate the nature of disease progression. At this time, there is no substitute for in vivo efficacy studies, studies to assess drug metabolism and pharmacokinetics. With this proposal, we have budgeted for the purchase of an IVIS Lumina III in vivo imager. This technology facilitates the monitoring of virus replication in live animals over time rather than the

traditional means of sacrificing multiple cohorts to gain similar data. In vivo imaging studies require fewer animals in step with the 3R principle (replacement, reduction and refinement). Our studies are designed with the fewest number of animals while retaining statistical significance.

3. Minimization of Pain and Distress

Rodents: SARS-CoV-MA15, MERS-15 and select bat coronaviruses replicate efficiently in the lungs of mice and may produce significant disease in young and aged animals, including acute onset respiratory distress syndrome, a clinically devastating end stage lung disease with 50% mortality rates. Mice will be closely monitored daily for signs of clinical disease. Since analgesics may affect the outcome of infections, analgesics will not be used and we will rely on close monitoring and euthanasia of sick animals to prevent undue pain and suffering. In general, animals will be euthanized if they approach losing 30% of their starting weight; we recognize that this is a significant weight loss, but acceptable as some animals can recover from >25% weight loss after highly pathogenic coronavirus infection. We will euthanize moribund animals, regardless of weight loss criteria. For rodents, euthanasia will be performed by overdose with isofluorane. This will immediately be followed organ harvest/exsanguinations, as prior treatment with these agents ensure that the animals will not suffer during this procedure due to operator error. This approach was chosen because unconsciousness and death occur quickly and the method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

Non-human Primates: Analgesics cannot be used on these animals because they might have unintended physiological effects that would influence pathogenesis and disease course, thereby interfering with the ability to detect protective effects of the drug treatment. An important strategy to minimize pain and distress is euthanasia of subjects as soon as scientific end-points are achieved. A clinical score sheet has been developed for NHPs to aid in the assessment of animal welfare. An overdose of anesthesia consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA) will be used to euthanize the animals. Deep anesthesia will first be induced in the animal using Telazol (5-7 mg/kg) prior to injection of a euthanizing agent. Death will be confirmed by the attending veterinarian by absence of a heartbeat and open chest examination.

10. Select Agent Research:

10a. Identify the Select Agent(s) to be used in the proposed research: We propose using Severe Acute Respiratory Syndrome-associated Coronavirus (SARS-CoV) and SARS-CoV genome RNA (isolated using Trizol) in this proposal. Derivative viruses encoding 2/3 genome length SARS-CoV are also considered as select agents.

10b. Provide the registration status of all entities where Select Agent(s) will be used: Both Vanderbilt University and The University of North Carolina at Chapel Hill are currently registered with the CDC for select agent use including SARS-CoV as required by select agent regulations (42 CFR 73).

10c. Provide a description of all facilities where the Select Agent(s) will be used: SARS-CoV will be

(b)(3):7 U.S.C. § 8401

10c(i). Describe the procedures that will be used to monitor possession, use and transfer of the Select Agent(s): (b)(3):7 U.S.C. § 8401

(b)(3):7 U.S.C. § 8401

10c(ii). Describe plans for appropriate biosafety, biocontainment, and security of the Select Agent(s):

(b)(3):7 U.S.C. § 8401

10c(iii). Describe the biocontainment resources available at all performance sites: (b)(3):7 U.S.C. § 8401

(b)(3):7 U.S.C. § 8401

(b)(3):7 U.S.C. § 8401

10c(iiii). **GOF Research**. Recognizing that US gain of function regulations (GOF) are under review, SARS-CoV and MERS-CoV are currently GOF pathogens and reverse genetic studies are subject to review. Our group has considerable expertise in interfacing with the appropriate NIH GOF institutional review boards to review, revise and finalize research designs that have the potential to modify pathogenesis or transmissibility in mammals. Our group has proposed experiments to introduce group 2b (SARS or SARS-like SHC014 or WIV1) or group 2c (MERS-CoV) S glycoprotein genes into the backbone of group 2d bat coronaviruses, enhancing chimeric virus replication in primary human airway cells and in mouse models of human disease. The purpose of these experiments is to demonstrate the ability of the Gilead drug to attenuate group 2d coronavirus pathogenesis and/or replication both in primary human cell cultures and in animal models of human disease. We and others note that coronaviruses are emerging pathogens. We and others also recognize that future group 2d pandemic zoonotic viruses may endanger human populations, thus known antivirals on a shelf can protect future generations from devastating disease outbreaks. Inclusion of the SARS or MERS S glycoprotein into group 2d bat coronavirus genomes is a potential GOF experiment that will definitely require review. As SHC014 and WIV1 are bat coronaviruses with an unknown capacity to produce pandemic disease or to encode increased transmissibility in humans, these chimeras likely fall outside of GOF consideration but still require review. We recognize that these group 2b SCH014 and WIV1 bat CoV spikes allow for use of the hACE2 receptor and program robust infections of primary human airway epithelial cells. If the proposal is successful, we will work in good faith with our program officer, local IBC, and the appropriate NIH subcommittee's to review, discuss and resolve GOF related issues associated with this proposal, ensuring safety and transparency for the greater public health.

11. Multiple PD/PI Leadership Plan

We have chosen a Multi-PI leadership plan for this project, as we believe the project will benefit from the shared leadership of two principal investigators with diverse expertise. Both Drs. Baric and Sheahan share a clearly defined goal of using synthetic genome design, primary human airway cells, in vivo imaging, improved small and large animal models of human disease, state of the art expertise in small molecule inhibitor design and improved clinically relevant therapeutic metrics to develop and evaluate GS-5734 prior to clinical testing. Dr. Baric is an expert at using reverse genetics platforms, metagenomics and synthetic genome design to recover recombinant viruses, using primary human airway cells to study emerging virus-host interactions and replication, and has developed robust small animal models of human disease. Dr. Sheahan has over a decade of experience in academic translational research with CoV and HCV and also has key industrial preclinical antiviral development experience gained while at GlaxoSmithKline. His understanding of academic and industrial enterprise has proven essential to the success of the current collaboration with Gilead Sciences. Drs. Baric and Sheahan will jointly interact with their collaborators, Drs. (b)(6); (b)(3):7 U.S.C. § 8401 to perform the in vitro primary human lung cell assays and with (b)(6); (b)(3):7 U.S.C. § 8401 at UTMB where the non-human primate studies will be performed. We note that Drs (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401

The research project will be organized as follows: Drs. Baric and Sheahan will jointly oversee all aspects of the research and administration associated with project. Dr. Baric will oversee all in vitro testing of the GS-5734 molecular in the proposal. Dr. Sheahan will be responsible for overseeing and performing all rodent model based in vivo studies. The key personnel on this project will meet weekly to discuss details of the experimental plan, progress, technical issues, data analysis, and interpretation. Strong collaborative relationships already exist between the research groups comprising this program and open and frequent lines of communication have already been established. Given their good working relationship, it unlikely that conflicts regarding scientific, fiscal, or regulatory matters will arise that cannot be resolved by the PIs. However, should these types of conflicts arise, they will first establish meetings with the entire program leadership (e.g. (b)(6); (b)(3):7 U.S.C. § 8401 (b)(6); (b)(3):7 U.S.C. § 8401 and the group of five will work to resolve the situation by majority vote. In parallel, we work in consultation with NIH Program Officials to discuss and resolve these issues. Should one of the co-directors not be able to full-fill their duties, then the remaining co-director will temporarily appoint another PI to perform the necessary duties and responsibilities until the leadership structure can be restored to normal.

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12. Consortium/Contractual Arrangements:

1. (b)(6); (b)(3):7 U.S.C. § 8401 of Vanderbilt University will serve as a key collaborator on this project. (b)(6); (b)(3):7 will generate resistance mutants to GS-5734, identify the mutations driving resistance using deep sequencing and use reverse genetics to introduce those mutations back into parental viruses to conclusively demonstrate specific genetic determinants that provide resistance to drug. (b)(6); (b)(3):7 U.S.C. § 8401 will also spearhead efforts to evaluate the impact of resistance mutations on virus replication and fitness in vitro, which will include assessment of cross sensitivity to other nucleoside analogs and performing competitive fitness assays. (b)(6); (b)(3):7 U.S.C. § 8401 will interface with the consortium members during a monthly conference call that will facilitate data sharing and adherence to milestones.

2. (b)(6); (b)(3):7 U.S.C. § 8401 of The University of Texas Medical Branch will perform drug efficacy and toxicity studies in non-human primates. (b)(6); (b)(3):7 U.S.C. § 8401 will assess the therapeutic efficacy of GS-5734 against MERS-CoV and assess the prophylactic and therapeutic efficacy of GS-5734 against SARS-CoV. Complete virological and pathological assessment will be performed on each animal including virus titers in multiple organs, histopathology, immunohistochemistry, CT-scan to assess pulmonary inflammation and pneumonia, and complete blood counts. (b)(6); (b)(3):7 U.S.C. § 8401 will interface with the consortium members during a monthly conference call that will facilitate data sharing and adherence to milestones.

GILEAD
Advancing Therapeutics
Improving Lives

Timothy Sheahan, Ph.D.
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September 16, 2016

Dear Tim,

I'm writing this letter in support of your grant proposal entitled "Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV" to be submitted for funding to the National Institute for Allergy and Infectious Diseases (RFA-AI-16-034). I am a Research Director in the department of Biology at Gilead Sciences where I manage a group of scientists responsible for discovery and development of antiviral compounds to treat acute virus infections.

Gilead is a leader in antiviral development with marketed products that have revolutionized the treatment of HIV, HBV, HCV, and influenza. Recently, Gilead has devoted resources to leverage our expertise in antivirals to develop therapeutics for the treatment of diseases caused by emerging and neglected viral pathogens. This program has led to the discovery of GS-5734, a nucleoside analog with broad spectrum activity being developed to treat Ebola virus infection. Based upon the data generated in your laboratory demonstrating that GS-5734 is also active against pathogenic coronaviruses such as MERS- and SARS-CoV, Gilead plans to file an Investigative New Drug (IND) application with the FDA to expand the indication for use of GS-5734 to treat MERS-CoV patients.

In order to develop drugs to treat emerging and neglected viral pathogens we require the help of good collaborators who have knowledge, expertise, and the necessary facilities to work with these pathogens. Thus, we are eager to work with your laboratory to develop GS-5734 to treat pathogenic coronavirus infections. Your proposed studies will support our development plan by providing key information on compound metabolism in tissues relevant to infection. In addition, the studies proposed to characterize the phenotypes of virus variants with reduced GS-5734 susceptibility will provide the foundation for our clinical virology program that will monitor for drug resistance during our clinical studies. While we have demonstrated protection from MERS-CoV disease in non-human primates (NPH) via prophylactic administration, your proposed therapeutic studies with MERS-CoV in NHP will provide key insights into the tractability of GS-5734 to treat ongoing MERS-CoV infections in humans.

Gilead has extensive experience in developing nucleoside analogs to treat viral infections. Our deep knowledge of these compounds will ensure our successful collaboration and help bring a much needed therapeutic to MERS-CoV patients. Gilead is committed to supporting any additional preclinical and clinical studies necessary to bring GS-5734 to market. We have funded the procurement for all transgenic mice thus far for UNC's preclinical evaluation of GS-5734. Additionally, we are the funding of the creation of a transgenic mouse line at Jackson Laboratories that will facilitate the comprehensive evaluation of MERS-CoV in vivo efficacy at UNC. Your work will provide the necessary preclinical support for ultimate licensure of GS-5734 for treatment of MERS-CoV infections. As part of this collaboration, our team looks forward to working closely with your group to provide advice and expertise on studies as well as GS-5734 and other reagents to ensure success of your program.

By combining our knowledge of drug development with you expertise in coronaviruses, we expect to bring GS-5734 to market for treatment of MERS-CoV patients.

We look forward to working with you on this exciting program.

(b)(6)

Robert Jordan, Ph.D.
Director, Biology
Gilead Sciences, Inc.

utmb Health
Microbiology & Immunology

(b)(6); (b)(3):7 U.S.C. § 8401

Dr. Ralph S. Baric
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Letter of Intent

Dear Dr. Baric,

I am pleased to express my intent to act as a sub-contractor on University of North Carolina's grant application to NIH in response to a RFA-AI-16-034: Partnerships for Countermeasures Against Select Pathogens for sponsoring the development of an antiviral agent as a medical countermeasure (MCM) against MERS-CoV and related emerging CoV.

Application Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV.

Project dates: June 1, 2017 – May 31, 2022

Project goal: The overall project aims to further develop a nucleoside analog, GS-5734, as a global countermeasure against the public treat of MERS-CoV and related emerging Pan-CoV an effective MCM against Pan-CoV, including MERS-CoV and related emerging highly pathogenic Pan-CoVs. Funding will cover the efficacy testing in small animal models and in non-human primates (NHP), in preparation for seeking an IND approval for clinical studies in human volunteers in the future.

Responsibilities: The University of Texas Medical Branch (UTMB Health) is tasked with preclinical testing of the prophylactic and therapeutic efficacy of this GS-5734 antiviral candidate in NHPs (rhesus macaques) against MERS-CoV, SARS-CoV, and, possibly, a bat-derived SARS-CoV-CoV (WVI-1). Abilities of GS-5734 to inhibit viral replication, attenuate or abolish interstitial pneumonia and pulmonary histopathology as well as other morbidity will be used as endpoints for assessing the efficacy against pan-CoV infections.

utmb Health
Microbiology & Immunology

Sincerely,

(b)(6)

(b)(6); (b)(3):7 U.S.C. § 8401

14. Resource Sharing Plan.

14a. Data Sharing Plan. We have a strong philosophy in favor of data sharing. In the event that any publications originating primarily from the cell cultures, such as methods development, result in gene expression data it will be standardized according to current conventions and deposited in public access databases, i.e., MIAME-compliant and deposited in GEO. Any quantitative sequencing analysis will be entirely open source and/or deposited in dBGaP so that results and analysis procedures are also publicly available. Links to our data will be included in any and all publications. We note that we make many protocols available on our website (http://www.med.unc.edu/cfpulmcenter/core-facilities/tc#protocols).

14b. Reagent Sharing Plan. To share resources with the academic research community, we will use the uniform Material Transfer Agreement (MTA), which basically acknowledges that the materials are proprietary to Institutions of the Cooperative Agreement and permitting their use in a manner that is consistent with the Bayh-Dole Act and NIH funding requirements. Our individual NIH research grants require that research be made available to the scientific community and public. The primary method of data sharing is through peer-reviewed publications in scientific journals and by presentation at scientific meetings. In addition, data and results created from NIH supported research will be submitted to NIH in annual progress reports per the terms and conditions of this award.

14c. Intellectual Property. Intellectual property agreements, identified during the course of this project, will be accomplished by negotiation in good faith among the institutions and inventors. Any intellectual property discussions will take place with all key personnel present and UNC Office of Technology and Development will assist the inventors in the production of the necessary documents, working with the particular institutions, legal firms and commercial interests. It is anticipated that companies and institutions will have access to these reagents by MTA (for research purposes) or by a license agreement to be negotiated in good faith with a company.

14d. Sharing Model Organisms. The $Ces1c^{-/-}/288/330^{+/+}$ mice will be a novel animal strain developed during this project. Gilead Sciences, Jackson laboratories and the Baric and Sheahan laboratories will work together to determine the appropriate MTA or paperwork required if requests are made to receive these animals. Model organisms such as cell lines, etc. and any useful unique reagent (cDNA's, vector constructs, etc.) that are generated and reported in publications have been, and will be, made fully available to all reasonable requestors in the scientific community. We have shipped cells worldwide.

14e. Genome-Wide Association Studies (GWAS). Not applicable.

15. Authentication of Key Biological and/or Chemical Resources.

15a. Cells.

Early passage primary lung cells from humans are a key reagent for the proposed studies. Human cells are derived from donors of both sexes and from all ages and ethnic groups. Care is taken during cell isolation to only handle one human organ at a time. Similarly, primary cell populations are handled carefully, only one donor cell type from a single donor at a time to avoid any mixing. The cells are observed to exhibit well-described prototypical characteristics of human primary lung cells in cell type specific medias in culture. For quality control, the cells are cultured in antibiotic free media to test for bacterial and fungal microbial contamination and are subjected to mycoplasma testing. Once the epithelial cells are grown as polarized and differentiated monolayers, a representative sample is subject to quality control histological analysis of cell morphology and Short Terminal Repeat (STR) marker profiling by the UNC Lineberger Cancer Center's Tissue Culture Facility (TCF). Routine evaluations for mycoplasma contamination are routinely performed in the laboratory.

- Certain experiments also employ immortal cell lines. Cell lines are obtained from the ATCC, or from the TCF. The TCF maintains cell lines, utilizing STR marker profiling and records of authentication are available. New cell lines not available directly from the TCF can be authenticated through the STR marker service provided by the TCF. Cell lines are routinely evaluated for mycoplasma contamination.
- When receiving cell lines, lab members initially maintain isolation and keep them isolated from other authenticated cell lines until mycoplasma testing and STR marker profiling is performed. All cell lines must be authenticated before commencing experimental work with them.

- Records are maintained for each of the cell lines regarding 1) the origin of the cell lines; 2) when they were resuscitated; 3) number of passages; 4) all test results; 5) any unique distinguishing growth behavior; and 6) any known genetic features.
- Cells that have been passaged for 6 months after receipt or from resuscitation will be re-authenticated, or a new vial of the working stock will be thawed.
- Lab members routinely examine cultured cell morphology by phase microscopy and monitor the growth characteristics in culture. New vials of the working stock are thawed if deviation from the baseline is observed.
- Mycoplasma contamination is re-checked whenever cells are extensively passaged to create new stocks.

15b. Animals (Mice)

- Rodent Genotyping. Mouse strain genetic validation. Inbred mouse strains are an invaluable tool for biomedical research, and represent a key aspect of this entire program. To ensure that the genetic background of all mice used within this program is known and when applicable they are part of a known inbred strains, we will genotype each mouse strain used within this program on the appropriate MUGA platform (Morgan, AP et.al., G3 2016, Dec 18). The most recent iteration of this state of the art genotyping array contains over 140,000 markers and can be used to precisely determine the genetic background at the substrain level and the precise location (at <1 megabase resolution) of genomic regions derived from different mouse inbred strains. In this way, the identity and genomic integrity of all mice used within these studies will be ensured. As new diagnostic assays become available, we will assess their utility and cost effectiveness the different MUGA arrays and implement them as appropriate.
- Furthermore, for each mutant mouse strain used within the project, positive diagnoses of the mutation will be assessed for each cohort of experimental animals with a diagnostic validated PCR assay or Sanger sequencing diagnostic to ensure proper results.

15c. Recombinant and Wildtype Viruses and Mutant Derivatives.

- Recombinant and wildtype viruses contain unique marker mutations that allow for distinguishing strains and mutation profiles, using a combination of full genome sequencing, reverse transcription-polymerase chain reaction (RT-PCR) or RT-PCR restriction fragment length polymorphism analyses (RT-PCR RFLP). Our group has developed defined primer pairs to distinguish between SARS-CoV and SARS-related bat coronaviruses as well as MERS-CoV and MERS-related bat coronaviruses. All viruses will be validated and certified pure of contaminating viruses prior to use or shipment to other laboratories.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Cells

- Early passage primary lung cells from humans are a key reagent for the proposed studies. Human cells are derived from donors of both sexes and from all ages and ethnic groups. Care is taken during cell isolation to only handle one human organ at a time. Similarly, primary cell populations are handled carefully, only one donor cell type from a single donor at a time to avoid any mixing. The cells are observed to exhibit well-described prototypical characteristics of human primary lung cells in cell type specific medias in culture. For quality control, the cells are cultured in antibiotic free media to test for bacterial and fungal microbial contamination and are subjected to mycoplasma testing. Once the epithelial cells are grown as polarized and differentiated monolayers, a representative sample is subject to quality control histological analysis of cell morphology and Short Terminal Repeat (STR) marker profiling by the UNC Lineberger Cancer Center's Tissue Culture Facility (TCF).
- Certain experiments also employ immortal cell lines. Cell lines are obtained from the ATCC, or from the TCF. The TCF maintains cell lines, utilizing STR marker profiling and records of authentication are available. New cell lines not available directly from the TCF can be authenticated through the STR marker service provided by the TCF.
- When receiving cell lines, lab members initially maintain isolation and keep them isolated from other authenticated cell lines until mycoplasma testing and STR marker profiling is performed. All cell lines must be authenticated before commencing experimental work with them.
- Records are maintained for each of the cell lines regarding 1) the origin of the cell lines; 2) when they were resuscitated; 3) number of passages; 4) all test results; 5) any unique distinguishing growth behavior; and 6) any known genetic features.
- Cells that have been passaged for 6 months after receipt or from resuscitation will be re-authenticated, or a new vial of the working stock will be thawed.
- Lab members routinely examine cultured cell morphology by phase microscopy and monitor the growth characteristics in culture. New vials of the working stock are thawed if deviation from the baseline is observed.
- Mycoplasma contamination is re-checked whenever cells are extensively passaged to create new stocks.

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/12/2018

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI132178-02
FAIN: R01AI132178

Principal Investigator(s):
Ralph S Baric (contact), PHD
Timothy Patrick Sheahan, PHD

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

Kati Chipps
104 Airport Drive
Suite 2200
Chapel Hill, NC 27599

Award e-mailed to: resadminosr@unc.edu

Period Of Performance:
Budget Period: 08/01/2018 – 07/31/2019
Project Period: 08/09/2017 – 07/31/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,166,670 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIV OF NORTH CAROLINA CHAPEL HILL in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI132178. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Laura A. Pone
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI132178-02

Award Calculation (U.S. Dollars)

Salaries and Wages	$154,747
Fringe Benefits	$46,236
Personnel Costs (Subtotal)	$200,983
Materials & Supplies	$220,895
Travel	$6,000
Other	$16,724
Subawards/Consortium/Contractual Costs	$471,000
Publication Costs	$2,000
Tuition Remission	$1,825
Federal Direct Costs	$919,427
Federal F&A Costs	$247,243
Approved Budget	$1,166,670
Total Amount of Federal Funds Obligated (Federal Share)	$1,166,670
TOTAL FEDERAL AWARD AMOUNT	$1,166,670
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,166,670

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$1,166,670	$1,166,670
3	$1,166,670	$1,166,670
4	$1,166,670	$1,166,670
5	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1566001393A1
Document Number:	RAI132178A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2018

IC	CAN	2018	2019	2020	2021
AI	8472315	$1,166,670	$1,166,670	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414E / **Released:** (b)(6) 07/11/2018
Award Processed: 07/12/2018 12:04:43 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI132178-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI132178-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as

those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI132178. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make

semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI132178-02

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **Vanderbilt University Medical Center.**

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This Notice of Award (NoA) includes funds for activity with **University of Texas Medical Branch.**

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Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Roberta D. Wolcott
Email: wolcottr@niaid.nih.gov **Phone**: 240-669-2964 **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI132178-02

INSTITUTION: UNIV OF NORTH CAROLINA CHAPEL HILL

Budget	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$154,747	$154,747	$154,747	$154,747
Fringe Benefits	$46,236	$46,236	$46,236	$46,236
Personnel Costs (Subtotal)	$200,983	$200,983	$200,983	$200,983
Materials & Supplies	$220,895	$220,895	$220,895	$220,895
Travel	$6,000	$6,000	$6,000	$6,000
Other	$16,724	$16,724	$16,724	$16,724
Subawards/Consortium/Contractual Costs	$471,000	$471,000	$471,000	$471,000
Publication Costs	$2,000	$2,000	$2,000	$2,000
Tuition Remission	$1,825	$1,825	$1,825	$1,825
TOTAL FEDERAL DC	$919,427	$919,427	$919,427	$919,427
TOTAL FEDERAL F&A	$247,243	$247,243	$247,243	$247,243
TOTAL COST	$1,166,670	$1,166,670	$1,166,670	$1,166,670

Facilities and Administrative Costs	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	55.5%	55.5%	55.5%	55.5%
F&A Cost Base 1	$445,482	$445,482	$445,482	$445,482
F&A Costs 1	$247,243	$247,243	$247,243	$247,243

A. COVER PAGE

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV	
Grant Number: 5R01AI132178-02	**Project/Grant Period:** 08/09/2017 - 07/31/2022
Reporting Period: 08/09/2017 - 07/31/2018	**Requested Budget Period:** 08/01/2018 - 07/31/2019
Report Term Frequency: Annual	**Date Submitted:** 06/14/2018
Program Director/Principal Investigator Information: RALPH S BARIC , PHD BS **Phone number:** (919) 966-3895 **Email:** rbaric@email.unc.edu	**Recipient Organization:** UNIV OF NORTH CAROLINA CHAPEL HILL UNIVERSITY OF NORTH CAROLINA CHAPEL HILL Office of Sponsored Research CHAPEL HILL, NC 275990001 **DUNS:** 608195277 **EIN:** 1566001393A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: R DAVID PAUL 104 Airport Dr. Suite 2200 Chapel Hill, NC 275991350 **Phone number:** 919-966-3411 **Email:** resadminosr@unc.edu	**Signing Official:** KATI CHIPPS 104 Airport Drive Suite 2200 Chapel Hill, NC 27599 **Phone number:** 9199624665 **Email:** kati_chipps@unc.edu
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1: Pharmacokinetics and Pharmacodynamics of GS-5734. 1) Synthetically reconstruct group 2D CoV. 2) Determine if antiviral effect and drug metabolism are equivalent in various primary cells targeted by SARS- and MERS-CoV through measurement of TP levels, virus replication and toxicity. 3) Create a transgenic model for MERS-CoV efficacy studies and assess efficacy in young and aged mouse models of SARS- and MERS-CoV disease. 4) Assess efficacy of GS-5734 in non-human primate models of SARS- and MERS-CoV

Aim 2: Defining Resistance to GS-5734 and Impact on Replication, Pathogenesis and Treatment. 1) Select MERS-CoV GS-5734 resistance mutants in continuous and primary human airway cells, and in wild-type animals. 2) Determine the effect of passage-selected reverse- engineered GS-5734 resistance mutations on replication fidelity, viral RNA synthesis, and competitive fitness as compared to wild-type parental virus. 3) Determine if resistance mutations alter viral replication, pathogenesis, or treatment in vivo.

Aim 3: Defining the Mechanism of Action of GS-5734. 1) In cell culture, determine if GS-5734 alters SARS- and MERS-CoV RNA synthesis, sequence diversity, and the innate immune response. 2) In mice, determine if GS-5734 alters SARS- and MERS-CoV RNA synthesis, sequence diversity, and the innate immune response. 3) Use RNA FISH to determine how drug affects viral RNA replication and the host response.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Opportunities.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

The results generated from this program have in part been disseminated via publication (See below) and will be presented at the International Conference on Antiviral Research (ICAR, Portugal 2018) (b)(6); as well as at the American Society for Virology (ASV) in College Park Maryland (2018) (b)(6); (b)(3):7. This work was also the subject of a presentation (Sheahan) at the North Carolina Museum of Natural Sciences “Going Viral” symposium. Public outreach is a priority for our team.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

vitro and in vivo studies comparing Kaletra/IFNb to GS-5734. Supplemental to this work, we aim to demonstrate GS-5734 efficacy in primary human T-cells which are targeted by MERS-CoV and represent an important extra-pulmonary cell population contributing to the overall presentation of disease. This collective work is targeted for submission for publication in Fall 2018. In the next reporting period, we will also progress our understanding of resistance generation through the phenotypic characterization of our passaged MERS-CoV and sequencing to identify mutations guiding resistance. The investigation into the mechanism of action of GS-5734 will also be a top priority. We have data suggesting that GS-5734 targets the viral nsp12 polymerase but how this drug interferes with replication remains unknown. We are using PCR, NGS and long-read RNA sequencing to test for GS-5734 incorporation, RNA premature termination, changes in defective genome formation, and other RNA modifications. The results may uncover novel strategies for GS-5734 CoV inhibition, as well as approaches to test the role of the identified resistance mutations. Our resistance studies suggest that nucleosides inhibitors may differentially target nsp12 polymerase and make the possibility of treatment with more than one nucleoside analog a more effective strategy. As a part of independently funded parallel studies, we are testing other nucleoside analogs to determine their efficacy against of WT MHV and MHV with resistance associated substitutions. We also have identified an addition mutation in MHV following selection with GS-5734, in the viral RNA helicase (nsp13). In the coming year we will test the impact of this substitution on GS-5734 resistance alone and in combination with the nsp12 polymerase V553L and F476L substitutions. We aim to develop a panel of CoV that represent family-wide genetic diversity to comprehensively assess spectrum breadth for antivirals against CoV. To this end, we will progress our efforts to create a bat group 2D recombinant infectious clone. Additionally, we will perform antiviral assays against other human CoV (OC43 and 229E) and a CoV with the most divergent nsp12, Porcine delta CoV (PDCoV), to determine if there are CoV nsp12s for which GS-5734 is no longer efficacious.

B.2 What was accomplished under these goals?

B.2.1. Overview of Major Activities and Industry Engagement. The overarching goal of our Partnership R01 Grant is to accelerate the preclinical development of GS-5734 (remdesivir) to support IND licensure for MERS-CoV for the continued progression towards human clinical trials. Thus, we work in close collaboration with Gilead Sciences. Through their consistent engagement in meetings/conference calls and reagent development/accessibility, Gilead Sciences has repeatedly demonstrated their commitment to this program. Additionally, they have funded several pharmacokinetic studies for our program and have even awarded our group short term supplemental funding prior this award for work that we had not proposed in this application. Given that our ultimate goal is to prepare GS-5734 for IND licensure and human clinical trial, we found it urgent to include a comparison to the current unofficial standard of care, Kaletra (HIV protease inhibitor)/Interferon-Beta (IFNb, an immunomodulator) currently being used in a clinical trial in the Kingdom of Saudi Arabia (KSA) (PMC5791210). Thus, the direct head to head comparison of Kaletra/Interferon-Beta and GS-5734 in both in cells in culture and in mice has been a top priority in Year 1 and will continue to be until complete.

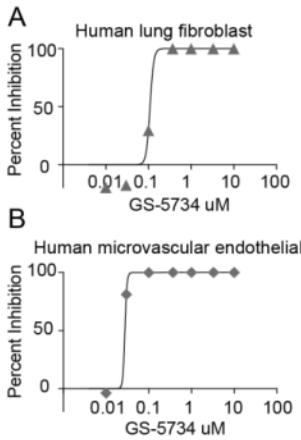

Figure 1: GS-5734 is potently antiviral in primary human lung fibroblasts and microvascular endothelial cells. Primary FB and MVE were infected with MERS at an MOI of 0.5 in the presence of GS-5734. Virus replication per condition was assessed via plaque assay.

B.2.2. Specific Objectives for Year 1. 1) Synthetically reconstruct group 2D CoV, 2) Assess efficacy in primary human lung cells, 3) Create a MERS-CoV mouse model to study GS-5734 efficacy, 4) Perform in vivo efficacy studies with GS-5734 against MERS-CoV, 5) Mechanism of action studies and resistance generation, 6) Demonstrate pharmacodynamic effect of IFNb treatment in mice**, 7) Head to Head Comparison of Kaletra/IFN-beta to GS-5734 cells and in mice**, and 8) NHP Model development for MERS-CoV. ** independently funded by Gilead Sciences

B.2.3. Significant Results for Year 1.

1. Synthetically reconstruct group 2D CoV. The coronavirus family can be divided into four genogroups (alpha, beta, gamma, delta). Group 2D thus far only contains bat CoV. Given the proclivity for bat CoV to emerge, we aim to determine GS-5734 efficacy against group 2D CoV. We are in the process of designing a molecular clone to generate recombinant virus for drug testing.

2. Assess efficacy in primary human cells. In collaboration with Dr. (b)(6); (b)(3):7 U.S.C. § 8401 an expert cell biologist specializing in primary human lung cell cultures at UNC, we have performed antiviral assays in multiple primary human lung cell types targeted by SARS- and MERS-CoV in the human lung. We have published our work in human airway epithelial cells (HAE, IC50 = 0.03μM). We have begun performing antiviral efficacy assays for MERS-CoV in human primary lung fibroblasts (FB) and primary microvascular endothelial cells (MVE) (**Fig. 1**) where we see submicromolar EC50 values in both types (FB IC50 = 0.1μM, MVE IC50 = 0.03μM).

3. A MERS-CoV mouse model to study GS-5734 efficacy. Since rodent orthologs of the human receptor, dipeptidyl peptidase 4 (DPP4) do not support MERS-CoV infection, we created a transgenic mouse via CRISPR/Cas9 to humanize two codons at positions 288 and 330 of mouse DPP4 (i.e. mDPP4 288/330+/+ mice) (PMC5578707). Unlike humans, mice express a secreted carboxylesterase 1c (Ces1c), which rapidly metabolizes GS-5734 in the blood before adequate distribution to target tissues. To circumvent this, in collaboration with Gilead Sciences we generated a Ces1c-/- and mDPP4 288/330+/+ (Ces1c-/-/288/330+/+) hybrid mouse colony for MERS-CoV efficacy studies.

4. In vivo efficacy studies with GS-5734 against MERS-CoV. Using our new MERS-CoV model and a dosing regimen proven to be effective for SARS-CoV, we have demonstrated that prophylactic GS-5734 (25mg/kg BID) can prevent severe lung disease caused by mouse adapted MERS-CoV (MERS P35C4) and significantly diminished virus replication (**Fig. 2**).

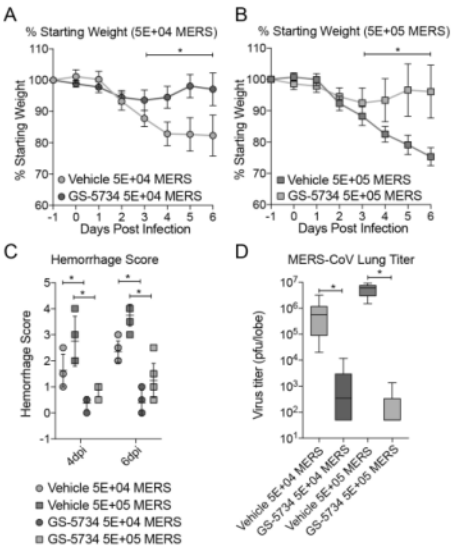

Figure 2: Prophylactic GS-5734 diminishes MERS-CoV disease and reduces virus replication. 9 to 12 week old Ces1c-/- hDPP4 mice were treated with vehicle or GS-5734 at 25mg/kg BID starting 24hr prior to infection with either 5E+04 or 5E+05 pfu MERS P35C4. **A** and **B)** Percent starting weight for 5E+04 and 5E+05 pfu MERS-CoV. **C)** Lung hemorrhage score. 0 is normal lung and 4 is 100% hemorrhaged. D) MERS-CoV lung titer 6dpi.

5. Mechanism of action studies and resistance generation. Through work spearheaded and recently published by Dr. (b)(6); (b)(3):7 U.S.C. § 8401 laboratory at Vanderbilt, we determined that passage of a murine CoV, MHV, selected for mutations in the nsp12 RNA dependent RNA polymerase at conserved residues that conferred up to 5.6-fold increase in GS-5734 EC_{50} but diminished replicative fitness is competition assays. Introduction of these resistance mutations into SARS-CoV transferred the resistance phenotype, and also attenuated SARS-CoV pathogenesis in mice. Further, an MHV mutant lacking nsp14 exoribonuclease (ExoN) proofreading was significantly more sensitive to GS-5734. Combined, the results demonstrate that GS-5734 interferes with the nsp12 polymerase even in the setting of intact CoV nsp14-ExoN proofreading activity. In addition, these studies demonstrate that resistance is difficult to select, only partial, and impairs fitness and virulence of MHV and SARS-CoV, providing critical new data to support further development of GS-5734 as a potential effective pan-CoV antiviral (Agostini et. al PMC5844999). Currently, we are passaging MERS-CoV in the presence of GS-5734 in HAE to determine if and how MERS-CoV generates drug resistance. GS-5734 has been proposed and with limited in vitro biochemical analysis to function as a non-obligate chain terminator, which should not show dose dependent decreased specific infectivity. We thus tested remdesivir along with other known mutagens (5-FU) and chain terminators (2'-C-meA) for the effects on MHV virion specific infectivity.

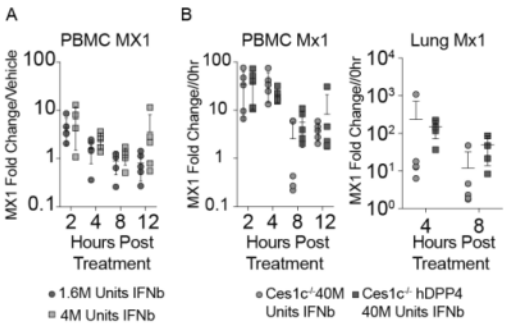

Figure 4: IFN stimulated gene MX1 is upregulated in mouse blood cells and lung after subcutaneous IFNb. A) Ces1c-/- mice were subcutaneously administered 1.6M or 4M units of IFNb. PBMCs were isolated over time for qRT-PCR analysis. B) Ces1c-/- or Ces1c-/- hDPP4 mice were subcutaneously administered 1.6M or 4M units of IFNb. PBMCs and lung tissue were isolated over time for qRT-PCR analysis. For both panels, total RNA was isolated for qRT-PCR for Mx1 and GAPDH.

(b)(4)

(b)(4)

(b)(4)

6. Pharmacodynamics (PD) of interferon beta treatment in mice. To prepare for our head to head comparison of GS-5734 to Kaletra (HIV protease inhibitor) and IFNb, Gilead Sciences designed and paid for two studies in Ces1c-/- or Ces1c-/- hDPP4 mice at a Contract Research Organization to understand the kinetics and magnitude of IFNb dosing in mice. We view Gilead support for this head to head comparison of GS5734 with the current lead treatment regimen in KSA an important development that will not only promote licensure and use in human trials but also because it led to supplementary support from our corporate partner. Mice were given a human equivalent dose (1.6 million units) or 2.5X or 25X the human equivalent dose of mouse IFNb and known interferon stimulated gene, MX1, expression was quantitated in lung and blood over time by qRT-PCR in our laboratory. We found a rapid dose dependent induction in MX1 expression in IFNb treated mice over control animals in both peripheral blood mononuclear cells as well as in lung tissue (**Fig. 4**).

7. Head to Head Comparison of Kaletra/IFN-beta to GS-5734 in cells and mice. It is imperative that GS-5734 be compared to current treatment options. Thus, we have performed head to head comparisons of Kaletra/IFNb and GS-5734 in human lung cells and in mice in studies funded by Gilead Sciences. To be completed in Year 2, we are now optimizing antiviral assays in the primary-like Calu-3 2B4 cell (human lung epithelial) to obtain IC50 values for each component of Kaletra (lopinavir/ritonavir) separately and together with and without IFNb. We are also performing head to head therapeutic efficacy studies in mice. We have been using human equivalent dosing for both Kaletra/IFNb and GS-5734. Our data thus far clearly demonstrates that Kaletra/IFNb does not reduce virus replication or disease. In contrast, as expected, we see a significant decrease in MERS-CoV viral load in the lung with GS-5734 treatment (**Fig 5.**)**.** With GS-5734, we were not able to protect against the development of disease likely due to the relatively high MERS-CoV inoculating dose (5E+05 pfu) that may be overwhelming the respiratory system in the 24hr prior to treatment. Future studies will utilize less input virus (5E+04 pfu).

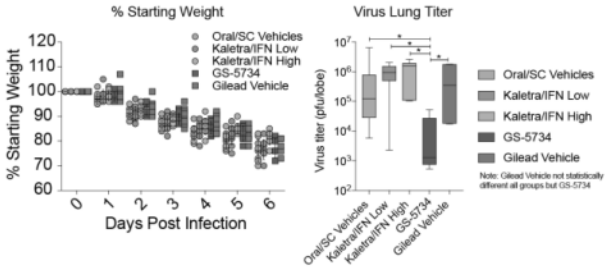

Figure 5: GS-5734 significantly reduces replication yet Kaletra/IFNb fails to diminish disease and virus replication. Percent starting weight is diminished similary for all groups while the virus lung titer is only significantly reduced with GS-5734.

8. Non-human primate (NHP) model development. Dr. (b)(6); (b)(3):7 U.S.C. § 8401, an investigator at the University of Texas Medical Branch and Galveston National Laboratories, will be leading our NHP efficacy efforts. Both MERS- (Rhesus macaque) and SARS-CoV (African green monkey) infection of NHP results in replication and mild disease. Moreover, Gilead has recently performed MERS-CoV prophylactic and therapeutic studies in Rhesus macaques at NIAID Rocky Mountain Laboratories thus completing the essential NHP efficacy studies. Since MERS-CoV replication is undetectable after 4 days and the disease does not reflect that seen in humans, there is a need for improved animal models. Thus, this past year, (b)(6); (b)(3):7 U.S.C. § 8401 has focused on developing strategies for NHP adaptation of SARS and MERS to be executed in year 2.

B.2.4. Key Outcomes or Other Achievements. We have made great progress in achieving our overarching goal to accelerate the preclinical development of GS-5734. We have a better understanding of its spectrum against CoV, its ability to ameliorate disease against multiple CoV in vivo and the capacity for CoV to generate drug resistance mutations. Due to additional funds from Gilead Sciences we shifted some of our priority in the first year to determine in vivo efficacy of GS-5734 against MERS-CoV in comparison to current therapeutics (Kaletra/IFNb). These data are essential for the progression of GS-5734 to human clinical trial. Our initial publication in Science Translational Medicine demonstrating that GS-5734 is a broad-spectrum antiviral against CoV continues to garner attention where it earned an Altmetric Score of 152 (Top 5% of all publications).

B.4 What opportunities for training and professional development has the project provided?
Postdoctoral fellows and graduate students are active in the project. Individual development plans (IDPs) are generated on an annual basis. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For this project, the IDPs will review specific goals relevant to the project. For postdoctoral fellows in addition they help in career development. For IDPs, both biosketches and CVs are generated, so that it is possible to use these as learning tools.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	(b)(6); (b)(3):7 U.S.C. § 8401
N/A: Not Peer Reviewed	
Complete	
In Process at NIHMS	Is regulation preventing the development of therapeutics that may prevent future coronavirus pandemics?. Future virology.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Models	We have developed the Ces1c-/- hDPP4 mouse model for the in vivo efficacy testing of GS-5734 with MERS-CoV. This model development was paid for by Gilead Sciences and performed at Jackson Laboratories. This model has yet to be published and is thus not available for sharing.

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b)(6); (b)(3):7 U.S.C. § 8401	Y	Baric, Ralph S	BS,PHD	PD/PI	(b)(4); (b)(6)					NA
	Y	Sheahan, Timothy Patrick	BS,PHD	PD/PI						NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		Technician						NA
	N		BA	Technician						NA
	N		PHD	Co-Investigator						NA
	N		PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N			Technician						NA
	N		PHD,MS	Staff scientist (Doctoral level)						NA
	N			Technician						NA
	N		PHD,MD, MS,BS	Co-Investigator						NA
	Y		MD	Co-Investigator						NA
	N			Undergraduate Student						NA
	N		BS	Graduate Student (research assistant)						NA
	N			Undergraduate Student						NA
	N		PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N		BS,PHD	Co-Investigator						NA
	N			Technician						NA
	Y		PHD,MS	Co-Investigator						NA

	N	(b)(6); (b)(3):7 U.S.C. § 8401		Technician	(b)(4); (b)(6)			NA

Glossary of acronyms:

S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: PartnershipOS.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

No

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 (b)(4)
NIH/NIAID $1,572,931
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-08/31/22 (b)(4)
NIH/NIAID $1,974,213
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

P01AI106695 00008956 (PI: Harris) 07/29/15-06/30/19 (b)(4)
UCB/NIH $279,165
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Co-Investigator

R01 AI 107731 (PI: De Silva) 08/05/13-07/31/18 (b)(4)
NIH/NIAID NCE
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4)
UAB/NIH/NIAID $304,371
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4)
Columbia/NIH/NIAID $584,891
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 (b)(4)
NIH/NIAID $609,870

Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Not Assigned (PI: Baric) 01/08/16-07/31/19 (b)(4)
(b)(4) $1,243,048
In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines
To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

R01 AI125198 (PI: deSilva) 05/04/16-04/30/21 (b)(4)
NIH/NIAID $1,153,997
Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines. Role: Co-Investigator

60045042 (PI: Baric) 02/01/15-01/31/19 (b)(4)
Ohio State Univ/USDA $44,804
Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs
Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.

64807 (PI: Baric) 06/23/16-06/22/18 (b)(4)
Takeda Vaccines, Inc $1,066,500
Breadth of Blockade Antibody Responses Following Norovirus Vaccination
To conduct a project as an agreement in which Dr. Ralph Baric will test Takeda provided serum samples for cross-strain blockade antibody responses.

N005402801 (PI: Li) 06/07/16-05/31/19 (b)(4)
Univ Minn/NIH $120,384
Receptor recognition and cell entry of coronaviruses
To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

Not Assigned (PI: Baric) 08/01/17-06/30/18 (b)(4)
Emory/NIH $96,463
Elucidating the potential of nucleoside analog, EIDD-1931, as a broad-spectrum antiviral against highly pathogenic human coronavirus strains
To define the activity, potency and mechanism of action of EIDD-1931 against highly pathogenic human coronaviruses for development as potential therapeutic.

R01AI127845 (PI: Becker-Dreps) 09/01/16-08/31/21 (b)(4)
NIH $506,771
Natural history, immunity, and transmission patterns of sapovirus in a Nicaraguan birth cohort
To characterize the natural history and risk factors for sapovirus gastroenteritis, elucidate the development of immunity to sapovirus in early childhood and the potential protective effect of maternal immunity, and apply novel genetic and analytic tools to characterize patterns of sapovirus transmission in households and communities. Role: Investigator

R01AI132178 (PI: Baric/Sheahan) 08/09/17-07/31/22 (b)(4)

NIH $1,184,372

Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

To focus on two areas: novel second generation compounds or compounds not previously provided by Gilead Sciences; and selecting and evaluating drug resistance profiles for SARS-CoV and MERS-CoV mutants in primary human lung cells. Role: Investigator

(b)(4)

R01 AI108197 (MPI: Denison/Baric) 04/01/18-03/31/23
Vanderbilt Univ/NIH $1,465,603

Determinants of Coronavirus Fidelity in Replication and Pathogenesis

To identify common and unique determinants of CoV nsp14-ExoN functions CoV replication, fidelity and IFN sensitivity across CoVs; To determine pathways of adaptation to loss of nsp14-ExoN activity in vitro and in vivo; and To define mechanisms of ExoN-regulated CoV sensitivity to the innate antiviral immune response. Role: MPI

(b)(4)

(b)(4) (PI: Brewer) 10/1/2017-9/30/2021
500,000 Euros

Why do norovirus pandemics occur and how can we control them?

The goal of this proposal is to determine the underlying evolutionary and molecular mechanisms governing the emergence of new norovirus pandemic strains, using a variety of cohort samples and novel diagnostic reagents.

(b)(4)

R21 AI137887 (MPI: Moorman/Heise) 02/05/18-01/31/20
NIH/NIAID $150,000

Molecular Characterization of Functional RNA Structures in the ZikV genome

Zika virus is an emerging pathogen that is associated with severe congenital neurologic defects, such as microcephaly. The proposed studies will identify new viral virulence determinants that can be targeted to generate safer and more effective Zika virus vaccines and therapeutics.

(b)(4)

R21 AI135682 (MPI: Geogiou/Baric) 02/01/18-1/31/20
NIH/NIAID $150,000

Molelcular Analysis of Serum Antibody Constituents in Zika Virus Infection

The goals of this project are to assess and quantify the persistence of specific and protective antibodies as well as cross-reactive (possible pathogenic) antibodies in Zika-infected patient blood. The antibodies developed in the project may lead to rapid development of new therapeutics and aid I n the design of future vaccine against Zika virus.

PENDING:

(b)(4)

OVERLAP: None

OTHER SUPPORT

SHEAHAN, TIMOTHY

ACTIVE:

(b)(4)

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator
Grants Management Specialist: Maureen Beanan NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: beananm@mail.nih.gov
Specific Aims Project 2: 1. To identify and develop inhibitors of CoV high-fidelity replication. 2. To identify and develop inhibitors of CoV RNA capping activity. 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors.

(b)(4)

R01 AI131688-01 (PI: Rice) 04/01/17-03/31/22
Rockefeller/NIH
Analysis of immunity, viral adaptation and pathogenesis in a new mouse model of HCV-related rodent hepacivirus infection
Mechanisms that contribute to the persistence of hepatotropic viruses, such as HCV, are not well understood. We have recently established the first immune-competent mouse model of an HCV-related virus. With this new model, we propose to systematically study immunity and host-virus interactions during a hepatotropic RNA virus infection in vivo.
Role: Co-Investigator
Grants Management Specialist: Rajen Koshy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: rkoshy@niaid.nih.gov
Specific Aims: Access to human liver tissue is limited. The only immunocompetent animal model of HCV infection, the chimpanzee, is no longer readily available for research. However, we have recently succeeded in establishing the first immune- competent mouse model of an HCV-related virus, Norway rat hepacivirus (NrHV). Our preliminary characterization of this model revealed significant virological and immunological similarities with HCV infection in humans. This advance now opens the opportunity to interrogate hepatic antiviral immunity, host-virus interactions, viral adaptation, immune evasion strategies and pathogenesis of a hepatotropic virus at an unprecedented level. In this proposal we plan to comprehensively analyze innate and adaptive intrahepatic immune responses during hepacivirus infection in vivo and to define determinants of viral clearance.

(b)(4)

1R01 AI132178-01 (MPI: Sheahan/Baric) 08/06/17-07/31/22
NIH $1,184,372
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
In partnership with Gilead Sciences, we aim to accelerate the preclinical development of GS-5734 and promote IND licensure. We define the pharmacokinetics, pharmacodynamics, resistance profile, efficacy breadth and mechanism of action of GS-5734 against MERS-CoV and related emerging CoV.
Grants Management Specialist: Erik Stemmy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: erik.stemmy@nih.gov
Specific Aims: In Aim 1, we refine the pharmacokinetics, pharmacodynamics and breadth of GS-5734 through efficacy and metabolism studies in various primary human cells with a diverse array of human and zoonotic CoV and through the evaluation of in vivo efficacy in murine and non-human primate models of MERS- and SARS-CoV. In Aim 2, we select for resistance against SARS-CoV and MERS- CoV, and determine the effect of resistance on virus replication, fitness and susceptibility to treatment. In Aim 3, we determine if the mechanism of action of GS-5734 is a result of direct effects on viral RNA replication and/or alteration of antiviral immunity via deep sequencing and single molecule RNA fluorescence in situ hybridization of vehicle or drug treated infected cells and mice.

(b)(4)

Not Assigned (PI: Baric) 08/01/17-06/30/18
Emory/NIH $96,463

Elucidating the potential of nucleoside analog, EIDD-1931, as a broad-spectrum antiviral against highly pathogenic human coronavirus strains

To define the activity, potency and mechanism of action of EIDD-1931 against highly pathogenic human coronaviruses for development as potential therapeutic.

(b)(4)

2R01 AI108197-06 (MPI: Baric/Denison) 03/01/18-02/28/23
Vanderbilt/NIH $1,465,603

Determinants of Coronavirus Fidelity in Replication and Pathogenesis

To identify common and unique determinants of CoV nsp14-ExoN functions CoV replication, fidelity and IFN sensitivity across CoVs; To determine pathways of adaptation to loss of nsp14-ExoN activity in vitro and in vivo; and to define mechanisms of ExoN-regulated CoV sensitivity to the innate antiviral immune response.
Role: Co-Investigator

Grants Management Specialist: Erik Stemmy NIAID, 5601 Fishers Lane, MSC 9806 Bethesda, MD 20892-9806 Email: erik.stemmy@nih.gov

OVERLAP:

If another application is funded, effort among the above projects will be adjusted such that the total effort does not exceed 100%.

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address

Primary: The University of North Carolina at Chapel Hill	608195277	NC-004	104 Airport Drive, CB 1350 Suite 2200 Chapel Hill NC 275991350
Vanderbilt University Medical Center	079917897	TN-005	1161 21st Avenue South D-7235 MCN Nashville TN 372322581
University of Texas Medical Branch	800771149	TX-014	301 University Blvd Galveston TX 775551070

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 07/15/2019

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Grant Number: 5R01AI132178-03
FAIN: R01AI132178

Principal Investigator(s):
Ralph S Baric (contact), PHD
Timothy Patrick Sheahan, PHD

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV

Kati Chipps
104 Airport Drive
Suite 2200
Chapel Hill, NC 27599

Award e-mailed to: resadminosr@unc.edu

Period Of Performance:
Budget Period: 08/01/2019 – 07/31/2020
Project Period: 08/09/2017 – 07/31/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $1,166,670 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIV OF NORTH CAROLINA CHAPEL HILL in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R01AI132178. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Tseday G Girma
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5R01AI132178-03

Award Calculation (U.S. Dollars)

Salaries and Wages	$154,747
Fringe Benefits	$46,236
Personnel Costs (Subtotal)	$200,983
Materials & Supplies	$220,895
Travel	$6,000
Other	$16,724
Subawards/Consortium/Contractual Costs	$471,000
Publication Costs	$2,000
Tuition Remission	$1,825
Federal Direct Costs	$919,427
Federal F&A Costs	$247,243
Approved Budget	$1,166,670
Total Amount of Federal Funds Obligated (Federal Share)	$1,166,670
TOTAL FEDERAL AWARD AMOUNT	$1,166,670
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$1,166,670

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$1,166,670	$1,166,670
4	$1,166,670	$1,166,670
5	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy and Infectious Diseases Research
CFDA Number:	93.855
EIN:	1566001393A1
Document Number:	RAI132178A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2019

IC	CAN	2019	2020	2021
AI	8472315	$1,166,670	$1,166,670	$1,166,670

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M51C B / **OC**: 414E / **Released**: (b)(6) 07/11/2019
Award Processed: 07/15/2019 12:03:27 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01AI132178-03

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5R01AI132178-03

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01AI132178. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made

publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5R01AI132178-03

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **Vanderbilt University Medical Center**

This Notice of Award (NoA) includes funds for activity with **University of Texas Medical Branch**

*****
Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.

*****
Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

- A list of the new and/or additional Agent(s) that will be studied;
- A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
- The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Mariama D. Diallo
Email: mariama.diallo@nih.gov **Phone**: 301-761-7851 **Fax**: 301-493-0597

Program Official: Erik J. Stemmy
Email: erik.stemmy@nih.gov **Phone**: 240-627-3380

SPREADSHEET SUMMARY
GRANT NUMBER: 5R01AI132178-03

INSTITUTION: UNIV OF NORTH CAROLINA CHAPEL HILL

Budget	Year 3	Year 4	Year 5
Salaries and Wages	$154,747	$154,747	$154,747
Fringe Benefits	$46,236	$46,236	$46,236
Personnel Costs (Subtotal)	$200,983	$200,983	$200,983
Materials & Supplies	$220,895	$220,895	$220,895
Travel	$6,000	$6,000	$6,000
Other	$16,724	$16,724	$16,724
Subawards/Consortium/Contractual Costs	$471,000	$471,000	$471,000
Publication Costs	$2,000	$2,000	$2,000
Tuition Remission	$1,825	$1,825	$1,825
TOTAL FEDERAL DC	$919,427	$919,427	$919,427
TOTAL FEDERAL F&A	$247,243	$247,243	$247,243
TOTAL COST	$1,166,670	$1,166,670	$1,166,670

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	55.5%	55.5%	55.5%
F&A Cost Base 1	$445,482	$445,482	$445,482
F&A Costs 1	$247,243	$247,243	$247,243

A. COVER PAGE

Project Title: Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV	
Grant Number: 5R01AI132178-03	**Project/Grant Period:** 08/09/2017 - 07/31/2022
Reporting Period: 08/01/2018 - 07/31/2019	**Requested Budget Period:** 08/01/2019 - 07/31/2020
Report Term Frequency: Annual	**Date Submitted:** 06/14/2019
Program Director/Principal Investigator Information: RALPH S BARIC , BS PHD **Phone number:** (919) 966-3895 **Email:** rbaric@email.unc.edu	**Recipient Organization:** UNIV OF NORTH CAROLINA CHAPEL HILL UNIVERSITY OF NORTH CAROLINA CHAPEL HILL Office of Sponsored Research CHAPEL HILL, NC 275990001 **DUNS:** 608195277 **EIN:** 1566001393A1 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: R DAVID PAUL 104 Airport Dr. Suite 2200 Chapel Hill, NC 275991350 **Phone number:** 919-966-3411 **Email:** resadminosr@unc.edu	**Signing Official:** KATI CHIPPS 104 Airport Drive Suite 2200 Chapel Hill, NC 27599 **Phone number:** 9199624665 **Email:** kati_chipps@unc.edu
Human Subjects: Yes HS Exempt: No Exemption Number: Phase III Clinical Trial:	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1: Pharmacokinetics and Pharmacodynamics of GS-5734. 1) Synthetically reconstruct group 2D CoV. 2) Determine if antiviral effect and drug metabolism are equivalent in various primary cells targeted by SARS- and MERS-CoV through measurement of TP levels, virus replication and toxicity. 3) Create a transgenic model for MERS-CoV efficacy studies and assess efficacy in young and aged mouse models of SARS- and MERS-CoV disease. 4) Assess efficacy of GS-5734 in non-human primate models of SARS- and MERS-CoV

Aim 2: Defining Resistance to GS-5734 and Impact on Replication, Pathogenesis and Treatment. 1) Select MERS-CoV GS-5734 resistance mutants in continuous and primary human airway cells, and in wild-type animals. 2) Determine the effect of passage-selected reverse- engineered GS-5734 resistance mutations on replication fidelity, viral RNA synthesis, and competitive fitness as compared to wild-type parental virus. 3) Determine if resistance mutations alter viral replication, pathogenesis, or treatment in vivo.

Aim 3: Defining the Mechanism of Action of GS-5734. 1) In cell culture, determine if GS-5734 alters SARS- and MERS-CoV RNA synthesis, sequence diversity, and the innate immune response. 2) In mice, determine if GS-5734 alters SARS- and MERS-CoV RNA synthesis, sequence diversity, and the innate immune response. 3) Use RNA FISH to determine how drug affects viral RNA replication and the host response.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Training opportunities.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

The results generated from this program have in part been disseminated via past publications (See below) and future publications of which we have three in revision currently. Results from this program have also been recently presented at the International Conference on Antiviral Research (ICAR, Baltimore 2019) (Sheahan), Respiratory Dart (Miami 2018, Baric) and at the International Society for Influenza and Other Respiratory Diseases (ISIRV-2018, Baltimore).

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the next reporting period, we will continue to accelerate the preclinical development of RDV. First, we aim to assemble and recover two different group 2D CoV recombinant viruses which will facilitate efficacy testing with RDV against this CoV subgenus. These viruses will further increase our ability to define the spectrum of antiviral activity against the CoV family. A major focus of the next reporting period will be identifying the genetic pathways of MERS-CoV RDV resistance, engineering these mutations back into our infectious clone and recovering recombinant potentially resistant virus for antiviral efficacy testing. We will determine if drug resistance is associated with a change in replicative fitness or pathogenesis and if in vivo efficacy is altered with resistant virus challenge. Relatedly, another major focus will be to further define the MOA of RDV for CoV using PCR, parallel deep- and direct RNA long-read sequencing, and virologic assays. We aim to determine how RDV affects CoV specific infectivity and the specific effects of RDV on CoV genomic and sub-genomic RNA. Using similar technologies, we will also initiate studies to determine if RDV alteration of viral RNAs leads to changes in host viral RNA sensing or initiation of the innate immune response. This work described in Aim 3 of our initial application will help further define the MOA of RDV. Lastly, we will initiate NHP studies aimed at creating better models of emerging CoV that will facilitate the evaluation of antiviral spectrum breadth.

B.2 What was accomplished under these goals?

B.2.1. Overview of Major Activities and Industry Engagement. The overarching goal of our Partnership R01 Grant is to accelerate the preclinical development of GS-5734 (**a.k.a. remdesivir, RDV**) to support IND licensure for MERS-CoV for the continued progression towards human clinical trials. Thus, we work in close collaboration with Gilead Sciences. Through their consistent engagement in meetings/conference calls, reagent development/accessibility and the funding of work prior to this award, Gilead Sciences has repeatedly demonstrated their commitment to this program. Given that our ultimate goal is to prepare RDV for IND licensure and human clinical trial, we found it urgent to compare RDV to a regimen (lopinavir/ritonavir and interferon beta, LPV/RTV+IFNb) currently under evaluation in human clinical trial in the Kingdom of Saudi Arabia (KSA) (PMC5791210). Thus, the direct head to head comparison of LPV/RTV+IFNb and RDV in both in cells in culture and in mice was the top priority in Year 2.

B.2.2. Specific Objectives for Year 2. 1) Design and synthetically reconstruct group 2D CoV, 2) Further evaluate antiviral activity spectrum against human and zoonotic CoV, 3) In vitro antiviral activity assays comparing RDV to LPV/RTV and IFNb, 4) Prophylactic and therapeutic efficacy studies in mice comparing RDV to LPV/RTV and IFNb, 5) Mechanism of action studies and resistance generation, 6) NHP model development.

B.2.3. Significant Results for Year 2.

1. Synthetically reconstruct group 2D CoV. The coronavirus family can be divided into four genera (1 (alpha), 2 (beta), 3 (gamma), 4 (delta)) and the 2D subgenus thus far only contains bat CoV. Given the proclivity for bat CoV to emerge, we aim to determine if RDV is efficacious against group 2D CoV. We have recently designed and ordered two infectious cDNA clones based on complete viral genome sequences isolated from fruit bats (*Rousettus leschenaulti*) in China. Once complete, we aim to recover these viruses as well as construct reporter virus versions. Recognizing that the group 2D genomes may prove replication competent, but unable to spread between cells because of Spike protein-receptor incompatibilities, we have also ordered and additional four group 2D spike glycoproteins, engineered to be inserted into either group 2D parental clone. This will increase our chances of recovering live infectious virus, especially if exogenous proteases enhance virion infectivity between cells as recently demonstrated by our group (Menachery et al., under review). Once characterized, antiviral assays will be developed for these viruses in order to determine the RDV antiviral activity against group 2D CoV.

2. Determine antiviral activity spectrum against human and zoonotic CoV. To further evaluate the spectrum of antiviral activity of RDV against the CoV family, we established antiviral assays for two endemic human CoVs OC43 (HCoV-OC43) and 229E (HCoV-229E) as well as the zoonotic porcine deltacoronavirus (PDCoV). For HCoVs-OC43 and -229E as well as PDCoV, RDV EC_{50} values were submicromolar. It is important to note that deltacoronavirus have the most divergent RNA dependent RNA polymerase (70% amino acid identity) as compared to SARS- and MERS-CoV of known CoVs (data not shown). These data further extend the known breadth and antiviral activity of RDV to include both contemporary human and highly divergent zoonotic CoV and potentially enhance our ability to fight future emerging CoV. A manuscript detailing this work is currently in revision at Antiviral Research.

3. Assess in vitro antiviral activity of lopinavir, ritonavir and interferon beta. Prior to embarking on comparative in vivo efficacy studies comparing lopinavir (LPV), ritonavir (RTV) and interferon beta (IFNb), we

first determined their antiviral activity against MERS-CoV in isolation and in combination. RDV showed potent inhibition of MERS-CoV replication with a EC_{50} of 0.09 µM and no observable cytotoxicity up to 10 µM in a human lung epithelial cell line (Calu-3). In contrast, the EC_{50} values for LPV (11.6 µM) and RTV (24.9 µM) were more than 2 logs greater than RDV. Interestingly, the EC_{50} for IFNb against MERS-CoV in Calu-3 was 175 IU/mL (CC_{50} > 2800 IU/mL) did not change significantly when coupled with a clinically relevant fixed concentration of LPV/RTV (5 µM:1 µM) (EC_{50} IFNb+LPV/RTV = 160 IU/mL), indicating IFNb likely to be the sole contributor to antiviral activity of the LPV/RTV+IFNb combination observed in vitro.

(b)(4)

(b)(4)

(b)(4)

5. Mechanism of action studies and resistance generation. To define the mechanism of action of RDV against CoV we have been pursuing complementary approaches at UNC and Vanderbilt University Medical Center (VUMC). At UNC, we have been passaging MERS-CoV in human primary airway epithelial (HAE) cultures in the presence of either high (1µM) or low (0.25µM) RDV. Upon quantitation of MERS-CoV titers from each passage, we found by passage 18, MERS-CoV titers were increased over 2 logs from those observed in passage 1 (**Fig. 2**). We are currently in the process of determining if passage 18 virus has increased resistance to RDV and are sequencing virus to determine if mutations guiding resistance have been accumulated. The goal is to determine if MERS-CoV can evolve resistance to RDV in a most biologically relevant human primary cell system. Additionally, since the initial resistance passaging was performed with mouse hepatitis virus (MHV), we aimed to determine if the pathways taken to achieve RDV resistance were similar or different among CoV.

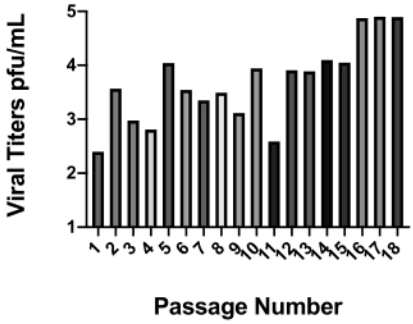

Figure 2: MERS-CoV virus production 4 days post infection in human airway epithelial cell cultures treated with 1µM RDV. For passage 1, cultures were infected at an MOI of 0.5 for 24hr prior to exposure to RDV. For passages, 2-10, cultures were infected with the previous passage for 24hr prior to exposure to RDV. For passages 11-18, infection with the previous passage and exposure to RDV were concurrent.

Work at VUMC have been focused on determining the mechanism of action of RDV against CoV. We previously observed that treatment with remdesivir resulted in a dose-dependent decrease in specific infectivity

(b)(4)

(PFU/particle ratio) of progeny MHV virions, a phenotype that has been linked with mutagenesis. This was surprising since remdesivir has been shown to function as a non-obligate chain terminator for respiratory syncytial virus (RSV) and Ebola virus and we did not expect that to impact specific infectivity, *a priori*. Therefore, we tested two other nucleoside analogs in this assay with disparate MOAs: a known mutagen (β-D-N4-hydroxycytidine; NHC) and chain terminator (2' -C-methyladenosine; 2'-C-MeA). (b)(4)

(b)(4)

(b)(4) we are combining PCR, parallel deep- and direct RNA long-read sequencing, and virologic assays to further dissect what may be a multi-modal MOA against CoVs.

Effect of remdesivir resistance-associated substitutions (RAS) on sensitivity to other nucleoside analogues. In our published studies, we showed that the mutations in the MHV nsp12 RNA-dependent RNA polymerase (V553L and F476L) acquired through passage in the presence of RDV conferred up to 6-fold increase in EC_{50}. To determine whether these mutations confer similar resistance in MERS-CoV, we engineered a recombinant MERS-CoV containing mutations at homologous positions (nsp12-F481L/V558L). While these mutations do not affect replicative fitness (**Fig. 2A**), (b)(4)

(b)(4)

(b)(4)

We previously showed that remdesivir RAS increase sensitivity of MHV to NHC (EIDD-1931), which acts as a mutagen. We next determined the sensitivity of MHV containing remdesivir RAS to 2'-C-MeA, which inhibits replication of other viruses by chain termination (**Fig. 3**). (b)(4)

(b)(4)

(b)(4)

6. Non-human primate (NHP) model development. Dr. Chieng Kent Tseng, an investigator at the University of Texas Medical Branch and Galveston National Laboratories (GNL), is leading our NHP efficacy efforts. Gilead has recently performed MERS-CoV prophylactic and therapeutic studies in rhesus macaques at NIAID Rocky Mountain Laboratories, thus completing the essential NHP efficacy studies for RDV preclinical development. There are no NHP models for zoonotic emerging CoVs similar to SARS-CoV (SCH014, WIV1) or MERS (HKU5, HKU4 etc.). Similarly, there are no NHP models for newly emerging pathogens like swine acute diarrhea syndrome (SADS-CoV) which have unknown epidemic potential in humans. Thus, this past year, Dr. Tseng has managed the repair of all equipment at GNL required for high quality NHP studies (i.e. x-ray, CT-scan, etc.). All of this equipment is now on-line. Thus, in the next year, we aim to design and execute the development of new CoV NHP models for the ultimate goal of creating better models to assess medical counter measures and the spectrum of efficacy against the CoV family.

B.2.4. Key Outcomes or Other Achievements. We have made great progress accelerating the preclinical development of RDV. We have a better understanding of its spectrum against CoV, its ability to ameliorate disease against multiple CoV in vivo and the capacity for CoV to generate drug resistance mutations. Thus, we continue to build a comprehensive preclinical data package to support IND licensure.

B.4 What opportunities for training and professional development has the project provided?
Postdoctoral fellows and graduate students are active in the project. Individual development plans (IDPs) are generated on an annual basis. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For this project, the IDPs will review specific goals relevant to the project. For postdoctoral fellows in addition they help in career development. For IDPs, both biosketches and CVs are generated, so that it is possible to use these as learning tools.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	(b)(6); (b)(3):7 U.S.C. § 8401
Complete	

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Models	(b)(4)

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
(b)(6); (b)(3):7 U.S.C. § 8401	Y	Baric, Ralph S	BS,PHD	PD/PI	(b)(4); (b)(6)					NA
	Y	Sheahan, Timothy Patrick	BS,PHD	PD/PI						NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	MS,PHD	Co-Investigator						NA
	N		MD	Co-Investigator						NA
	N		PHD,MD, MS,BS	Co-Investigator						NA
	N		PHD	Co-Investigator						NA
	N		PHD,MS	Staff scientist (Doctoral level)						NA
	N			Technician						NA
(b)(6); (b)(3):7 U.S.C. § 8401	N		PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	BS	Graduate Student (research assistant)						NA
	N			Undergraduat e Student						NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401		Technician						NA
	N			Technician						NA
	N		BA	Technician						NA
	N			Technician						NA
	N			Technician						NA
	N		BS	Technician						NA
(b)(6); (b)(3):7 U.S.C. § 8401	N		BS,PHD	Co-Investigator						NA
	N		PHD,MS	Co-Investigator						NA

(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401	PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	(b)(4); (b)(6)			NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

File uploaded: Other_Support.pdf

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OTHER SUPPORT

BARIC, RALPH S.

ACTIVE-SUBJECT AWARD

R01AI132178 (PI: Baric/Sheahan) 08/09/17-07/31/22 (b)(4)
NIH $919,427
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
To focus on two areas: novel second generation compounds or compounds not previously provided by Gilead Sciences; and selecting and evaluating drug resistance profiles for SARS-CoV and MERS-CoV mutants in primary human lung cells.

ACTIVE:

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-08/31/22 (b)(4)
NIH/NIAID $2,662,979
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/20 (b)(4)
UAB/NIH/NIAID $375,233
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/29/20 (b)(4)
Columbia/NIH/NIAID $165,767
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

00008956 (PI: Desilva) 07/29/15-06/30/19 (b)(4)
UCB/NIH $183,021
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Investigator

R01-AI125198 (PI: DeSilva) 05/04/16-04/30/21 (b)(4)
NIH/NIAID $846,094
Preclinical Assays To Predict Tetravalent Dengue Vaccine Efficacy
Dengue is the most significant mosquito transmitted viral infection of humans. Vaccination is a feasible solution to prevent and control dengue. Although dengue vaccines are under development, we do not know the specific properties of antibodies induced by vaccines that are likely to protect from infection. In this project investigators from the University of North Carolina and Sanofi Pasture, a leading dengue vaccine developer, will collaborate to define properties of antibodies induced by the Sanofi vaccine that correlate with protection. The main goal of the project is to develop new assays to support the current global effort to develop dengue virus vaccines.
Role: Investigator

(b)(4) (PI: Desilva) 06/30/14-12/31/19 $699,504 (b)(4)

UNC (b)(4) study to characterize human antibody response to DENVax

The deSilva and Baric laboratories will jointly characterize the properties of neutralizing antibodies in the serum samples using competition assays with monoclonal antibodies and neutralization assays with recombinant viruses. Role: Investigator

R01 AI110700 (PI: Baric) 04/20/15-03/31/20 $605,933 (b)(4)
NIH/NIAID

Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis

The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

(b)(4) (PI: Baric) 01/08/16-07/31/19 $1,243,048 (b)(4)

In Vitro and In Vivo Characterization of Bivalent DENV Live Virus Vaccines

To provide expertise in molecular virology required for creating recombinant dengue viruses for in vitro and in vivo testing.

R21 AI135682 (MPI: Geogiou/Baric) 02/01/18-1/31/20 $191,625 (b)(4)
NIH/NIAID

Molelcular Analysis of Serum Antibody Constituents in Zika Virus Infection

The goals of this project are to assess and quantify the persistence of specific and protective antibodies as well as cross-reactive (possible pathogenic) antibodies in Zika-infected patient blood. The antibodies developed in the project may lead to rapid development of new therapeutics and aid I n the design of future vaccine against Zika virus.

R01 AI108197 (MPI: Denison/Baric) 03/01/18-02/28/23 $532,971 (b)(4)
Vanderbilt Univ/NIH

Determinants of Coronavirus Fidelity in Replication and Pathogenesis

To identify common and unique determinants of CoV nsp14-ExoN functions CoV replication, fidelity and IFN sensitivity across CoVs; To determine pathways of adaptation to loss of nsp14-ExoN activity in vitro and in vivo; and To define mechanisms of ExoN-regulated CoV sensitivity to the innate antiviral immune response.

R01AI127845 (PI: Becker-Dreps) 09/27/16-08/31/21 $500,513 (b)(4)
NIH

Natural history, immunity, and transmission patterns of sapovirus in a Nicaraguan birth cohort

To characterize the natural history and risk factors for sapovirus gastroenteritis, elucidate the development of immunity to sapovirus in early childhood and the potential protective effect of maternal immunity, and apply novel genetic and analytic tools to characterize patterns of sapovirus transmission in households and communities. Role: Investigator

N005402801 (PI: Li) 06/07/16-05/31/21 $120,384 (b)(4)
Univ Minn/NIH

Receptor recognition and cell entry of coronaviruses

To investigate how CoVs explore host receptors and host proteases for regulation of their host range, cross-species transmission, tissue tropism, and pathogenesis. Role: Subcontract PI

D43 TW010923 (PI: Becker-Dreps/Meshnick) 05/10/18-02/28/23 $230,000 (b)(4)
NIH

Nicaraguan Emerging and Endemic Diseases (NEED)

The goals of this program are to 1) train young Nicaraguan scientists in Infectious Disease Epidemiology at the UNC, 2) create a sustainable supply of scientists in the region by establishing an accredited PhD program in Biomedical Sciences at the Universidad Nacional Autonoma de Nicaragua Leon and 3) foster

professional growth and development among trainees and local faculty to ensure academic and research success. Role: Investigator

R21 AI137887 (MPI: Moorman/Heise) 02/05/18-01/31/20 (b)(4)
NIH/NIAID $150,000
Molecular Characterization of Functional RNA Structures in the ZikV genome
Zika virus is an emerging pathogen that is associated with severe congenital neurologic defects, such as microcephaly. The proposed studies will identify new viral virulence determinants that can be targeted to generate safer and more effective Zika virus vaccines and therapeutics. Role: Investigator

R01AI107731 (Desilva) 08/05/13-08/31/23 (b)(4)
NIH/NIAID $421,235
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
To determine the origin and properties of these useful cross protective antibodies. We will also study the properties of antibodies that neutralize Zika viruses.
Role: Investigator

K24AI141744 (Becker-Dreps) 12/06/18-11/30/23 (b)(4)
NIH/NIAID $157,100
The Development of Norovirus Immunity in Early Childhood and Implications for Norovirus Vaccines
To acquire new research skills and carry out a research plan that will allow guidance of the development of pediatric norovirus vaccines.
Role: Investigator

U01 AI149644 (PI: Baric) 04/19/19-03/31/24 (b)(4)
NIH/NIAID $644,071
Respiratory Virus Vaccine and Adjuvant Exploration
This project takes advantage of expertise in adjuvant development, vaccinology, and complex trait genetics, proposes to use advanced Systems Vaccinology and Genetics approaches to define the polymorphic genes/gene networks that regulate the immune response to select respiratory virus adjuvanted immunogens.

TERMINATED
U19 AI 107810 (PI: Baric) 06/21/13-05/31/19 (b)(4); (b)(6)
NIH/NIAID $1,766,262
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

PENDING:

(b)(4)

OVERLAP: None

OTHER SUPPORT

SHEAHAN, TIMOTHY

ACTIVE – SUBJECT AWARD

R01 AI132178-01 (MPI: Sheahan/Baric) 08/09/17-07/31/22 (b)(4)
NIH $919,427
Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV
In partnership with Gilead Sciences, we aim to accelerate the preclinical development of GS-5734 and promote IND licensure. We define the pharmacokinetics, pharmacodynamics, resistance profile, efficacy breadth and mechanism of action of GS-5734 against MERS-CoV and related emerging CoV.

ACTIVE

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/20 (b)(4)
UAB/NIH/NIAID $375,233
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease. Role: Investigator

R01 AI131688-01 (PI: Rice) 03/15/17-02/281/22 (b)(4)
Rockefeller/NIH $64,182
Analysis of immunity, viral adaptation and pathogenesis in a new mouse model of HCV-related rodent hepacivirus infection
Mechanisms that contribute to the persistence of hepatotropic viruses, such as HCV, are not well understood. We have recently established the first immune-competent mouse model of an HCV-related virus. With this new model, we propose to systematically study immunity and host-virus interactions during a hepatotropic RNA virus infection in vivo. Role: Subcontract PI

R01AI108197-06 (MPI: Baric/Denison) 03/01/18-02/28/23 (b)(4)
Vanderbilt/NIH $532,971
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
To identify common and unique determinants of CoV nsp14-ExoN functions CoV replication, fidelity and IFN sensitivity across CoVs; To determine pathways of adaptation to loss of nsp14-ExoN activity in vitro and in vivo; and to define mechanisms of ExoN-regulated CoV sensitivity to the innate antiviral immune response.
Role: Co-Investigator

U01AI149644 (PI: Baric) 04/19/19-03/31/24 (b)(4)
NIH $644,071
Respiratory Virus Vaccine and Adjuvant Exploration
Vaccination is one of the most effective public health measures for protecting against infectious disease, and the proposed studies will identify adjuvants and adjuvant combinations that safely elicit long lived protective immunity against emerging pathogens in at risk populations. Role: Investigator

TERMINATED

(b)(4) (PI: Sims) 06/07/17-06/06/18 (b)(4)
$120,000
Testing of (b)(4) lucleoside Analog Compounds
The overall goal of this project is to test (b)(4) protease inhibitor/interferon cocktails in comparison to and with nucleoside analog compounds to determine the best course of treatment for patients infected with highly pathogenic human coronaviruses.

Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/18 (b)(4)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

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E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Yes

Is the research exempt from Federal regulations?

No

Does this project involve a clinical trial?

No

G.4.b Inclusion Enrollment Data

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

No

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

No

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address

Primary: The University of North Carolina at Chapel Hill	608195277	NC-004	104 Airport Drive, CB 1350 Suite 2200 Chapel Hill NC 275991350
Vanderbilt University Medical Center	079917897	TN-005	1161 21st Avenue South D-7235 MCN Nashville TN 372322581
University of Texas Medical Branch	800771149	TX-014	301 University Blvd Galveston TX 775551070

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

Grant Number: 5U19AI109680-03 REVISED
FAIN: U19AI109680

Principal Investigator(s):
Richard J. Whitley, MD

Project Title: Antiviral Drug Discovery and Development Center - Overall

Shaun Pryor
Dir, Ofc of Sponsored Progs
Univ of Alabama at Birmingham
AB 1170
701 20th Street South
Birmingham, AL 352940111

Award e-mailed to: OSP-NGA@mail.ad.uab.edu

Period Of Performance:
Budget Period: 03/01/2016 – 02/28/2017
Project Period: 03/01/2014 – 02/28/2019

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF ALABAMA AT BIRMINGHAM in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number U19AI109680. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Regina E. Kitsoulis
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

SECTION I – AWARD DATA – 5U19AI109680-03 REVISED

Award Calculation (U.S. Dollars)

Salaries and Wages	$135,055
Fringe Benefits	$42,599
Personnel Costs (Subtotal)	$177,654
Consultant Services	$12,500
Materials & Supplies	$17,069
Travel	$51,808
Other	$25,583
Subawards/Consortium/Contractual Costs	$7,205,406
Federal Direct Costs	$7,490,020
Federal F&A Costs	$133,769
Approved Budget	$7,623,789
Total Amount of Federal Funds Obligated (Federal Share)	$7,623,789
TOTAL FEDERAL AWARD AMOUNT	$7,623,789
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
3	$7,623,789	$7,623,789
4	$7,293,471	$7,293,471
5	$7,112,904	$7,112,904

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Allergy, Immunology and Transplantation Research
CFDA Number:	93.855
EIN:	1636005396A6
Document Number:	UAI109680A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017	2018
AI	8023357	$472,974	$157,657	
AI	8472315	$7,150,815	$7,135,814	$7,112,904

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: M65B B / **OC**: 414P / **Released**: (b)(6) 08/24/2016
Award Processed: 08/25/2016 12:01:41 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5U19AI109680-03 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 5U19AI109680-03 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.

c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

Carry over of an unobligated balance into the next budget period requires Grants Management Officer prior approval.

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) U19AI109680. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award is funded by the following list of institutes. Any papers published under the auspices of this award must cite the funding support of all institutes.

National Institute Of Allergy And Infectious Diseases (NIAID)

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make

semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI Special Terms and Conditions – 5U19AI109680-03 REVISED

REVISED AWARD: This award is revised to process an internal Common Accounting Number (CAN) correction. All previous terms and conditions of award remain in effect.

Supersedes previous Notice of Award dated **06/24/2016**.
****

THIS AWARD CONTAINS GRANT SPECIFIC RESTRICTIONS. THESE RESTRICTIONS MAY ONLY BE LIFTED BY A REVISED NOTICE OF AWARD.

REVISED AWARD: This award provides supplemental funds of **$472,974** Total Costs (**$285,000** Direct Costs and **$187,974** F&A Costs) for **Zika Supplement/ Administrative Supplement**. These funds provide support for the period **06/01/2016 - 02/28/2017**. These funds are restricted for stated purpose, in request dated **04/01/2016**,from **Richard Whitley and Stephanie May / University of Alabama at Bitmingham**, and may not be rebudgeted or used for any other purpose, without NIAID awarding unit approval. Future year's supplemental funds (**$157,657** Total Costs; **$95,000** Direct Costs, **$62,657** F&A Costs) are also restricted.

Supersedes previous Notice of Award dated **02/05/2016**.
****

Based on the on the progress report submitted on **12/16/2015**, the following personnel will be committed over 12 Calendar Months (CM) with the awarding of this grant:

Michael Diamonds
JL Smith

The grantee institution is responsible for adjusting the effort as needed so that at no time the above named individual(s) totaleffort exceeds 12 CM.
****

This award includes funds awarded for subrecipient activity with **Southern Research Institute** in the amount of **$4,067,681** (**$1,963,588**direct costs + **$2,104,093**facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **Oregon Health and Science University** in the amount of **$1,076,614** (**$636,994**direct costs + **$439,620**facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **Vanderbilt University** in the amount of **$404,625** (**$257,723** direct costs + **$146,902** facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **The University of North Carolina at Chapel Hill** in the amount of **$690,644** (**$454,371** direct costs + **$236,273** facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **Washington University** in the amount of **$262,806** (**$172,332** direct costs + **$90,474** facilities and administrative costs).

This award includes funds awarded for subrecipient activity with **University of Colorado at Denver** in the amount of **$230,062** (**$150,091** direct costs + **$79,971** facilities and administrative costs).

Consortiums are to be established and administered as described in the NIH Grants Policy Statement (NIH GPS). The referenced section of the NIH Grants Policy Statement is available at http://grants.nih.gov/grants/policy/nihgps/HTML5/section_15/15_consortium_agreements.htm.
****

This award is issued as a Cooperative Agreement, a financial assistance mechanism in which substantial NIH scientific and/or programmatic involvement is anticipated in the performance of the activity. This award is subject to the Terms and Conditions of Award as set forth in Section VI: Award Administrative Information of **RFA AI-12-044**, "**Centers of Excellence for Translational Research (CETR) (U19)**," posted date **11/23/12**, which are hereby incorporated by reference as special terms and conditions of this award. [If applicable please add: These special Terms and Conditions of Award were included on the award notice for the **-01** year issued on **02/12/14**.]

This RFA may be accessed at: http://grants.nih.gov/grants/guide/index.html
****

Awardees who conduct research involving Select Agents (see 42 CFR 73 for the Select Agent list; and 7 CFR 331 and 9 CFR 121 for the relevant animal and plant pathogens at http://www.selectagents.gov/Regulations.html) must complete registration with CDC (or APHIS, depending on the agent) before using NIH funds. No funds can be used for research involving Select Agents if the final registration certificate is denied.

Prior to conducting a restricted experiment with a Select Agent or Toxin, awardees must notify the NIAID and must request and receive approval from CDC or APHIS.
****

Select Agents:
Awardee of a project that at any time involves a restricted experiment with a select agent, is responsible for notifying and receiving prior approval from the NIAID. Please be advised that changes in the use of a Select Agent will be considered a change in scope and require NIH awarding office prior approval. The approval is necessary for new select agent experiments as well as changes in on-going experiments that would require change in the biosafety plan and/or biosafety containment level. An approval to conduct a restricted experiment granted to an individual cannot be assumed an approval to other individuals who conduct the same restricted experiment as defined in the Select Agents Regulation 42 CFR Part 73, Section 13.b (http://www.selectagents.gov/Regulations.html).

Highly Pathogenic Agent:
NIAID defines a Highly Pathogenic Agent as an infectious Agent or Toxin that may warrant a biocontainment safety level of BSL3 or higher according to the current edition of the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm). Research funded under this grant must adhere to the BMBL, including using the BMBL-recommended biocontainment level at a minimum. If your Institutional Biosafety Committee (or equivalent body) or designated institutional biosafety official recommend a higher biocontainment level, the highest recommended containment level must be used.
When submitting future Progress Reports indicate at the beginning of the report:

If no research with a Highly Pathogenic Agent or Select Agent has been performed or is planned to be performed under this grant.

If your IBC or equivalent body or official has determined, for example, by conducting a risk assessment, that the work being planned or performed under this grant may be conducted at a biocontainment safety level that is lower than BSL3.

If the work involves Select Agents and/or Highly Pathogenic Agents, also address the following points:

Any changes in the use of the Agent(s) or Toxin(s) including its restricted experiments that have resulted in a change in the required biocontainment level, and any resultant change in location, if applicable, as determined by your IBC or equivalent body or official.

If work with a new or additional Agent(s)/Toxin(s) is proposed in the upcoming project period, provide:

o A list of the new and/or additional Agent(s) that will be studied;
o A description of the work that will be done with the Agent(s), and whether or not the work is a restricted experiment;
o The title and location for each biocontainment resource/facility, including the name of the organization that operates the facility, and the biocontainment level at which the work will be conducted, with documentation of approval by your IBC or equivalent body or official. It is important to note if the work is being done in a new location.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Jorge E. Machuca
Email: jorge.machuca@nih.gov **Phone**: 240-669-2981 **Fax**: 301-493-0597

Program Official: Maureen J. Beanan
Email: beananm@mail.nih.gov **Phone**: 240-292-0999

SPREADSHEET SUMMARY
GRANT NUMBER: 5U19AI109680-03 REVISED

INSTITUTION: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Budget	Year 3	Year 4	Year 5
Salaries and Wages	$135,055	$116,955	$116,955
Fringe Benefits	$42,599	$35,494	$35,494
Personnel Costs (Subtotal)	$177,654	$152,449	$152,449
Consultant Services	$12,500	$20,000	$10,000
Materials & Supplies	$17,069	$22,100	$22,100
Travel	$51,808	$58,000	$55,000
Other	$25,583	$30,619	$30,619
Subawards/Consortium/Contractual Costs	$7,205,406	$6,877,214	$6,715,757
TOTAL FEDERAL DC	$7,490,020	$7,160,382	$6,985,925
TOTAL FEDERAL F&A	$133,769	$133,089	$126,979
TOTAL COST	$7,623,789	$7,293,471	$7,112,904

Facilities and Administrative Costs	Year 3	Year 4	Year 5
F&A Cost Rate 1	47%	47%	47%
F&A Cost Base 1	$284,614	$283,168	$270,168
F&A Costs 1	$133,769	$133,089	$126,979

A. OVERALL COVER PAGE

Project Title: Antiviral Drug Discovery and Development Center - Overall	
Grant Number: 5U19AI109680-03	**Project/Grant Period:** 03/01/2014 - 02/28/2019
Reporting Period: 03/01/2015 - 02/29/2016	**Requested Budget Period:** 03/01/2016 - 02/28/2017
Report Term Frequency: Annual	**Date Submitted:** 12/16/2015
Program Director/Principal Investigator Information: RICHARD J WHITLEY , MD AB **Phone number:** 205-934-5316 **Email:** rwhitley@peds.uab.edu	**Recipient Organization:** UNIVERSITY OF ALABAMA AT BIRMINGHAM UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave South BIRMINGHAM, AL 352331806 **DUNS:** 063690705 **EIN:** 1636005396A6 **RECIPIENT ID:**
Change of Contact PD/PI: N/A	
Administrative Official: RICHARD B MARCHASE 701 20th Street South, AB1170 Birmingham, AL 352940111 **Phone number:** 2059345266 **Email:** osp@uab.edu	**Signing Official:** SHAUN R PRYOR 701 20th Street South AB 1170 Birmingham, AL 35294 **Phone number:** 2059662395 **Email:** spryor6@uab.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The past 15 years have witnessed the emergence and re-emergence of several human viral infections of life threatening proportions, including diseases attributable to SARS coronavirus, highly pathogenic H5N1 influenza, pandemic 2009 influenza, monkeypox imported into the United States (US), West Nile virus (WNV) and dengue. Arguably, no efficacious therapy exists for most of these diseases and resistance is a threat to circulating influenza. Experimental approaches have been applied to each one of these diseases but with varying degrees of success.

The goal of this program is to form the Antiviral Drug Discovery and Development Center (AD3C) and identify compounds working though mechanisms that affect viral RNA replication and, importantly, to develop these leads in a translational manner to new human therapeutics. All four projects in this program are focused on viruses deemed critical to NIAID's focus on Emerging and Re-emerging Infectious Diseases related to biodefense. The projects perform High Throughput Screening utilizing unique compound libraries to identify novel chemical scaffolds with antiviral activity. Importantly, the projects report strong preliminary data that demonstrate the feasibility of performance of proposed mechanistic analysis of inhibitory compounds. In addition, all projects already have existing active compounds that will enter the drug discovery and development pathway at a later stage for evaluation.

The common theme of our application is targeting viral RNA replication. The experimental strategies designed by the four projects will provide a comprehensive analysis of the mechanism of action of the potential hit compounds. For example, it has been known for a long time that there are four consensus sequences that are conserved among the RNA-dependent RNA polymerases encoded by plus, minus and double stranded RNA viruses (1). The novel drug libraries with their diverse functionalities will allow the identification of compounds that might target conserved regions of the polymerase and thus yield broad-spectrum antiviral compounds. Based on the existing data in the literature and the preliminary data generated in the laboratories of the four groups we hypothesize that the development of drugs, which target enzymes such as polymerase and 2'O-methyl-transferase are rational approaches for the treatment of these viral diseases and will be more effective than targeting the surface glycoproteins. Resistance to drugs targeting the glycoproteins has frequently been reported. We hypothesize that viral escape mutants resulting from drugs targeting polymerase will be unfit for RNA replication, based on recent data in the literature. This data demonstrated that the mechanism of activity by the reported T-705 anti-polymerase drug is by inducing lethal mutagenesis in the polymerase protein, resulting in a nonviable virus unfit for replication. In AD3C, we will combine the virus-specific knowledge of leading virologists in the world with the high throughput screening and medicinal chemistry and lead optimization capabilities of Southern Research. The program's general specific aims are thus to:

1.Test viral targets essential to RNA replication in high-throughput-screening assays with unique chemical libraries to establish lead molecules for drug discovery.
2.Validate lead compounds in secondary and tertiary assays to confirm selectivity and mechanism of action as well as assure absence of off-target effects.
3.Probe the effects of lead molecules in representative animal models of targeted diseases and utilize such data to define impact on disease pathogenesis. Medicinal chemistry will optimize leads and further define platforms.

The individual projects all follow the general approach as described above, and are led by Drs. Jay Nelson (OHSU) and Michael Diamond (Washington University) to study compounds active against flaviviruses; Drs (b)(6); (b)(3):7 (Vanderbilt) and Ralph Baric (UNC – Chapel Hill) to study compounds active against SARS-coronavirus; Drs. Dan Streblow (OHSU) and Mark Heise (UNC – Chapel Hill) to study compounds active against alphavirus and Drs. Ghalib Alkhatib, Jim Noah (Southern Research) and Rich Whitley (UAB) to study compounds active against influenza. All projects will extensively utilize the Screening Core and Medicinal Chemistry and Lead Development Core at Southern Research, which, with seven FDA approved drugs, has an outstanding track record of bringing drug discovery programs to clinical reality. All this will be coordinated out of the Administrative Core at UAB, which has extensive experience in drug discovery programs.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Major Goals Yr3 Umbrella
Year 3 will see a significant shift, away from identification of hit molecules, to the identification of lead molecules in all the Projects, with the help of the Cores. The analogues of either hits coming out of the HTS campaigns from Yr1 and Yr2 or from previously run campaigns should lead to identification of tractable chemical series, with appropriate drug-like properties in all 4 projects in the coming project period. Molecules will be tested not only in the Structure-Activity-Relationship (SAR-) driving assay in Core B, but also in the Projects in secondary and tertiary assays, and the mechanism of action of active compounds will start to be investigated.

As active molecules with acceptable profiles in Projects are being identified, we will continue and expand on the testing of these molecules against the multiple virus families in the various Projects, to identify broad-spectrum antiviral candidates.

Also, as we identify compounds with favorable in vitro pharmacokinetic (PK) properties, we will test in vivo PK and start to test molecules in relevant animal models.
Per the EAB recommendation, we will closely monitor the progress of the various chemical series, to identify non-productive avenues to improve potency or other drug-like properties and shift resources away from those series to other, more promising ones.

Although not part of the funded research under the U19, we did want to highlight that we will capitalize on the work directed against MERS through collaborations with BARDA; they will aid in assessing our lead molecule, provided by Gilead Sciences, in the marmoset model at the NIAID Rocky Mountain Laboratories. They will also assist with further optimization of the optimal dose in normal human volunteer studies, based on the pharmacokinetic properties.

IDPs:

Please refer to the Project descriptions for Individual Development Plans used by the respective institutions with which the trainees are affiliated.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not Peer Reviewed	Smith EC, Sexton NR, Denison MR. Thinking outside the triangle: Replication fidelity of the largest RNA viruses. Annual review of virology. 2014; 1:7.1-7.11.
Complete	Chiang C, Beljanski V, Yin K, Olagnier D, Ben Yebdri F, Steel C, Goulet ML, DeFilippis VR, Streblow DN, Haddad EK, Trautmann L, Ross T, Lin R, Hiscott J. Sequence-Specific Modifications Enhance the Broad-Spectrum Antiviral Response Activated by RIG-I Agonists. J Virol. 2015 Aug;89(15):8011-25. PubMed PMID: 26018150; PubMed Central PMCID: PMC4505665.
Complete	Broeckel R, Haese N, Messaoudi I, Streblow DN. Nonhuman Primate Models of Chikungunya Virus Infection and Disease (CHIKV NHP Model). Pathogens. 2015 Sep 16;4(3):662-81. PubMed PMID: 26389957; PubMed Central PMCID: PMC4584280.
PMC Journal - In process	Long KM, Ferris MT, Whitmore AC, Montgomery SA, Thurlow LR, McGee CE, Rodriguez CA, Lim JK, Heise MT. Gamma-delta T cells play a protective role in chikungunya virus-induced disease. J Virol. 2015 Oct 21;PubMed PMID: 26491151.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

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C.5.b Resource sharing

NOTHING TO REPORT

C.5.a Other Products

Reagents generated by Project 1:

1. **293T-IFIT1**: In Project 1.2 (Diamond), we have generated the doxycycline inducible 293T cell that expresses IFIT1. Upon publication, we will deposit this cell line at BEI Resources (ATCC) for use by the greater scientific community.

Reagents generated by Project 3:

2. **THF-ΔIRF-3:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells constitutively express the reverse Tet-transactivator via lentivector (Clontech # 631069); not relevant for this study but just FYI. The IRF3 gene sequence has been disrupted using the CRISPR/Cas9 system (AddGene vector # 49535). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell line constructed by Dr. DeFilippis.
3. **THF-ΔIFIT1, THF-ΔIFIT2, THF-ΔSTING, THF-ΔIPS1, THF-ΔSTAT1:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These are also stably transduced with a firefly luciferase-coding region under the control of the interferon responsive element using a lentivector obtained from System Biosciences. Individual cell lines were constructed in which the protein coding regions for IFIT1, IFIT2, STING, IPS1, or STAT1 were disrupted using the CRISPR/Cas9 system (AddGene vector # 52961). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell lines constructed by Dr. DeFilippis.
4. **CHIKV Caribbean Strain Infectious Clone:** $CHIKV_{99659}$ was recently isolated from the British Virgin Islands in December of 2013. A low-passage stock of this strain was provided to the members of the Alphavirus group from Dr. Michael Diamond (Project 2). The Heise lab, in collaboration with Dr. Nathanial Moorman at UNC, has sequenced the isolate and constructed an infectious clone of the virus.
5. **$CHIKV_{181/25}$ Strains Expressing nano-Luciferase (nLuc):** Into the infectious clone of $CHIKV_{181/25}$ was introduced an in-frame nLuc reporter gene. Two different viruses were constructed by the Heise Lab: pTH1.2 (NSP-3nLuc) and pTH2.1 (Capsid-nLuc), which will be utilized by SR for cherry-pick validation screens and for mechanism of action studies.
6. **$CHIKV_{AF15561}$ strain expressing mKate:** An in-frame mKate reporter gene was cloned into the infectious clone of the pathogenic parental virus of $CHIKV_{181/25}$ ($CHIKV_{AF15561}$). Constructed by Dr. Morrison's group.
7. **G10:** A novel small molecule (4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide) capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	SSN	DOB	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Component(s)	Country	SS
(b)(6)	Y	Whitley, Richard J.	(b)(6)	(b)(6)	AB,MD	PD/PI	(b)(4); (b)(6)						NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Non-Student Research Assistant					Project-5320 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	Everts, Maaike		(b)(6)	PHD	Co-Investigator					Admin Core-5318 (Administrative Core - Core A)		NA
	N	Hancock, Meaghan H			BS	Staff scientist (Doctoral level)					Project-5319 (Project 1.1 Identification …ug Candidates)		NA
	N	Maddadi, Nikhil				Technician					Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Martinez, Yohanka			MS	Technician					Project-5330 (Project 4.2 Identification …ase functions)		NA
	Y	PRICHARD, MARK Neal	(b)(6)	(b)(6)	PHD,BS, MS	Co-Investigator					Project-5322 (Project 4.1 Identification …ase functions)		NA
	N	Quenelle, Debra			DVM,PhD	Co-Investigator					Project-5322 (Project 4.1 Identification …ase functions)		NA
	N	Quick, Eric				Technician					Project-5330 (Project 4.2 Identification …ase functions)		NA
	N	Rice, Terri				Non-Student Research Assistant					Project-5322 (Project 4.1 Identification …ase functions)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PhD	Staff scientist (Doctoral level)					Project-5328 (Project 3.2 Novel Therapeu…		NA

										Alphaviruses)		
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PHD	Co-Investigator	(b)(4); (b)(6)			Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
	Y	Augelli-Szafran, Corinne				Co-Investigator				Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				Project-5328 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6)			PHD,BS	Graduate Student (research assistant)				Project-5329 (Project 3.3 Novel Therapeu… Alphaviruses)		NA
	Y	DeFilipis, Victor Robert	(b)(6)	(b)(6)	PHD,MS	Co-Investigator				Project-5321 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Ahmad, Fahim				Postdoctoral Scholar, Fellow, or Other Postdoctoral Position						NA
	Y	Hirsch, Alec	(b)(6)	(b)(6)	BA,PHD	Co-Investigator				Project-5319 (Project 1.1 Identification …ug Candidates)		NA
	Y	Smith, Jessica L			PHD	Co-Investigator				Project-5319 (Project 1.1 Identification …ug Candidates)		NA
	N	Kocher, Jacob			PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position				Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401				Co-Investigator				Project-5330 (Project 4.2 Identification …ase functions)		NA

(b)(6)	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PhD	Staff scientist (Doctoral level)	(b)(4); (b)(6)		Project-5328 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	Y	Suto, Mark J			BS,PHD	Co-Investigator			Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401			Ph.D.	Co-Investigator			Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Austin, Stephen Kyle			BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-5319 (Project 1.1 Identification …ug Candidates)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	N	(b)(6); (b)(3):7 U.S.C. § 8401			BS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position			Project-5320 (Project 2.1 Inhibitors of … Therapeutics)		NA
	Y	Sheahan, Timothy P.			PhD	Co-Investigator			Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Ahmed, Kaleem S				Chemist			Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Ando, Takeshi	(b)(6)		MD/PhD	Adj. Research Associate Professor			Project-5321 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	Bao, Donghui				Research Scientist			Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Bonin, Kiley	(b)(6)	(b)(6)		Non OHSU Student Worker			Project-5321 (Project 3.1 Novel Therapeu… Alphaviruses)		NA

	N	Bowers, Mary Wyatt	(b)(6)		MA	Admin Core Business Manager	(b)(4); (b)(6)		Admin Core-5318 (Administrative Core - Core A)		NA
	N	Cabrera, Sara		(b)(6)	M.S.F.S.	Supervisor Compound Management			Core-5323 (Screening Core - Core B)		NA
	N	Crawford, Christine			BS	Research Assistant			Project-5319 (Project 1.1 Identification …ug Candidates)		NA
	N	Davis, Sara				Admin Core Administrative Coord.			Admin Core-5318 (Administrative Core - Core A)		NA
	N	Denton, Michael	(b)(6)	(b)(6)	BS	Sr. Research Assistant			Project-5321 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			B.A.	Advanced Biologist			Core-5323 (Screening Core - Core B)		NA
	N	Keith, Kathy				Research Lab Supervisor			Project-5322 (Project 4.1 Identification …ase functions)		NA
	N	Kezar, Hollis				Chemist			Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Kreklywich, Nicholas	(b)(6)	(b)(6)		Non OHSU Student Worker			Project-5321 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Non-Student Research Assistant			Project-5320 (Project 2.1 Inhibitors of … Therapeutics)		NA
	N	Manuvakhova, Anna	(b)(6)	(b)(6)	B.S.	Advanced Bio IT Specialist			Core-5323 (Screening Core - Core B)		NA
	N	May, Nick				Professional Research Assistant			Project-5329 (Project 3.3 Novel		NA

											Therapeu… Alphaviruses)		
	N	Mitchell, Jennifer	(b)(6)	(b)(6)	MS	Research Assistant	(b)(4); (b)(6)						NA
	N	Moukha-Chafiq, Omar				Chemist					Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Nikhil, Maddadi				Chemist					Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Parkins, Christopher	(b)(6)	(b)(6)	M.S.	Research Associate							NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			M.S.	Supervisor HTS Cente					Core-5323 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			B.S.	Associate Biologist					Core-5323 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			B.S.	Associate Biologist					Core-5323 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			B.S.	Biologist					Core-5323 (Screening Core - Core B)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Research Technician					Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	Streblow, Aaron				Non OHSU Student Worker							NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	M.S.	Research Scientist					Core-5323 (Screening Core - Core B)		NA
	N	Truss, Jackie W				Chemist					Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	N	Vibha, Pathak				Chemist							NA

	N	Watterson, Zoe	(b)(6)	(b)(6)		Non-student worker	(b)(4); (b)(6)		Project-5319 (Project 1.1 Identification …ug Candidates)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401				Research Specialist			Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
	N	(b)(6); (b)(3):7 U.S.C. § 8401			M.S.	Biologist			Core-5323 (Screening Core - Core B)		NA
	N	Zhang, Wei				Research Scientist			Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
(b)(6)	N	Schaefer, Alexandra	(b)(6)	(b)(6)	Ph.D.	Research Associate			Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401			BS,PHD	Faculty			Project-5320 (Project 2.1 Inhibitors of … Therapeutics)		NA
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401			MD	Project 2.1 Project Leader			Project-5320 (Project 2.1 Inhibitors of … Therapeutics)		NA
	Y				BA,PHD	Project 3.2 Project Leader			Project-5328 (Project 3.2 Novel Therapeu… Alphaviruses)		NA
	N	Maddry, Joseph A			PhD	Medicinal Chemistry Core C Project Leader					NA
	Y	(b)(6); (b)(3):7 U.S.C. § 8401			PHD,BS	Screening Core B Project Leader					NA
	Y	White, E. Lucile			BA	Screening Core B Co-Project Leader			Core-5323 (Screening Core - Core B)		NA
	Y	Diamond,			PHD,MD	Project 1.2			Project-5325		NA

(b)(6)		Michael S			,BA,MD, PHD	Project Leader	(b)(4); (b)(6)		(Project 1.2 Identification …ug Candidates)		
(b)(6); (b)(3):7 U.S.C. § 8401	Y	(b)(6); (b)(3):7 U.S.C. § 8401	(b)(6)	(b)(6)	PHD,MS ,DVM	Project 4.2 Project Leader			Project-5330 (Project 4.2 Identification …ase functions)		NA
	Y	Nelson, Jay A			BS,PHD, BS,BOT H	Project 1 Leader			Project-5319 (Project 1.1 Identification …ug Candidates)		NA
	Y	Pathak, Ashish Kumar			PHD,MS ,BS	Advanced Research Scientist			Core-5324 (Medicinal Chemistry and Le…Core - Core C)		NA
	Y	Baric, Ralph S			PHD,BS	Project 2.2 Project Leader			Project-5327 (Project 2.2 Inhibitors of … Therapeutics)		NA
	Y	Streblow, Daniel N			PHD,BS	Project 3.1 Project Leader			Project-5321 (Project 3.1 Novel Therapeu… Alphaviruses)		NA
	Y	Morrison, Thomas E			MA,PHD ,BA,BA	Project Leader 3.3			Project-5329 (Project 3.3 Novel Therapeu… Alphaviruses)		NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

Yes

File uploaded: New Version Umbrella new key personnel.pdf

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

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D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

There are new senior/key personnel involved in the U19 in the following Core and Projects; their Biosketches and Other Support documents are appended after this page, in the order listed below.

Core C: Medicinal Chemistry and Lead Development Core

- Ashish K. Pathak

Because of the (b)(6) of Dr. Joseph Maddry, there is a now a need for change of key personnel from Dr. Joseph Maddry to Dr. Ashish Pathak. Dr. Pathak has been involved with the program since its inception and has been responsible for the majority of the ongoing chemistry efforts. Therefore, because of his familiarity with the program, and that his chemistry group has already been working on the program, it is a very smooth transition for this change in personnel. In addition, the experience and wealth of knowledge that Dr. Pathak has in medicinal chemistry as well as in antiviral research adds significance to his new role.

Project 2: Coronaviruses

- Timothy P. Sheahan

Project 4: Influenza

- (b)(6); (b)(3):7 U.S.C. § 8401
-

Because of the departure of Dr. Alkhatib from SR, (b)(6); (b)(3):7 U.S.C. § 8401 have taken over the direction and management of the influenza project at SR.

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Pathak, Ashish Kumar

eRA COMMONS USER NAME (credential, e.g., agency login): (b)(6)

POSITION TITLE: Advanced Research Scientist/Principal Investigator

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Lucknow, Lucknow, India	B.Sc.	06/1985	Chemistry, Physics, Mathematics
University of Lucknow, Lucknow, India	M.Sc.	07/1987	Organic Chemistry
University of Lucknow, Lucknow, India	Ph.D.	02/1993	Organic Chemistry

A. Personal Statement

The goal of the Medicinal Chemistry Core in the Antiviral Drug Discovery and Development Center (AD3C) at the University of Alabama at Birmingham (UAB) is to develop novel small molecule therapeutics for emerging and re-emerging viral infections against Dengue viruses (DENV), West Nile virus (WNV), Severe acute respiratory syndrome virus (SARS-CoV), Chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV) and Influenza viruses using high-throughput screening (HTS) on library of 300K+ compounds followed by medicinal chemistry. I have the expertise, leadership and motivation necessary to successfully carry out the proposed work and have a broad background in organic chemistry, with specific training and expertise in key research areas for this application. At various positions at research laboratories in India, Japan and here in USA, I carried out research in various aspect of organic/medicinal chemistry and specifically in synthetic carbohydrate chemistry and small molecule drug discovery. As a principal synthetic chemist on several previous university- and NIH-funded grants and as a PI on two R21 grants, I laid the groundwork for several funded research projects and also specifically to this proposed research project. My research team at Southern Research (SR) has worked in the past on semi-synthetic development of *Q.* saponins preparation GPI-0100 for Galenica Pharmaceuticals and for development of saponin based adjuvants for Marburg virus vaccine preparation. Currently my independent research group at SR executes internally and externally funded projects in the area of small molecule drug discovery, saponins as immune stimulants and carbohydrate synthesis. I am also the scientist responsible for parallel synthesis laboratory at SR. As an Assistant Professor, I supervised several research students leading to their MS degree in chemistry at Western Illinois University. In addition, I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced 55 peer-reviewed publications and 6 patent applications. As a result of these previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. I have extensive experience in supervising and executing intramural and extramural research projects. In summary, I have a demonstrated record of successful and productive research projects in an area of high relevance, and my expertise and experience have prepared me to be Medicinal Core PI in AD3C.

1. C.W. Evans, C. Atkins, A.K. Pathak, B.E. Gilbert, J.W. Noah*. Benzimidazole analogs inhibit respiratory syncytial virus G protein function. ***Antiviral Res.*** 121, 31-38 (2015). PMID: 26116756

2. A.K. Pathak,* J.A. Benitez, A.J. Silva-Benitez. Small molecule inhibitors of bacterial motility and a high throughput screening assay for their identification. US Patent 8,940,740 dated 2015/1/27.
3. B. Severson, D.H. Chung, C.B. Jonsson, E.L. White, L. Rasmussen, C.B. Maddox, S. Ananthan, A.K. Pathak, J.A. Maddry. Anti-viral treatment and assay to screen for anti-viral agent. International Publication No. WO/2011/097607A1.
4. A.K. Pathak,* V. Pathak and R.D. May. Adjuvant. US Patent No. 8,883,170B2 dated 2014/11/11.
5. D.J. Marciani*, R.C. Reynolds, A.K. Pathak, K. Finley-Woodman and R.D. May. Fractionation, structural studies, and immunological characterization of the semi-synthetic Quillaja saponins derivative GPI-0100. *Vaccine* 21, 3961-71 (2003). PMID: 12922132

B. Positions and Honors

Positions and Employment

2013-	**Advanced Research Scientist**, Chemistry Department, Drug Discovery Division, Southern Research Institute, Birmingham, AL.
2011-	**Adjunct Professor**, Department of Chemistry, University of Alabama at Birmingham (UAB), Birmingham, AL.
2009-2013	**Research Scientist**, Chemistry Department, Drug Discovery Division, Southern Research Institute, Birmingham, AL.
2005-2008	**Assistant Professor**, Department of Chemistry, Western Illinois University, Macomb, IL.
2000-2005	**Research Scientist**, Medicinal Chemistry Group, Drug Discovery Division, Southern Research Institute, Birmingham, AL.
1997-2000	**Research Associate**, Medicinal Chemistry Group, Drug Discovery Division, Southern Research Institute, Birmingham, AL.
1996-1997	**Senior Research Associate**, Central Institute of Medicinal and Aromatic Plants, Council of Scientific and Industrial Research (CSIR), India.
1995-1996	**Science and Technology Agency Fellow** (STA), National Institute of Health Sciences, Tokyo.
1993-1995	**Research Associate Fellow**, Central Institute of Medicinal and Aromatic Plants, Council of Scientific and Industrial Research (CSIR), India.
1992-1993	**Senior Research Fellow**, Central Institute of Medicinal and Aromatic Plants, Council of Scientific and Industrial Research (CSIR), India.

Other Experiences and Professional Memberships

2002 -	Member, American Chemical Society
2005 - 2008	Member, Arts & Science College Faculty Council Committee, Western Illinois University, Macomb, IL
2007 - 2008	Senator, WIU Faculty Senate, Western Illinois University, Macomb, IL
2013 - 2013	Mail Reviewer, multi-project grant applications RFA-AI-12-048, "Immune Mechanisms of Virus Control (U19)". NIAID Immune Mechanisms of Virus Control Program (IMVC), NIH
2014 - 2014	Reviewer, Contract BAA-NIHAI2013168: Adjuvant Discovery Program (2014), NIAID - ZAI1 QV-I (C1), NIH

Honors

1991	Senior Research Fellow, Council of Scientific and Industrial Research (CSIR), New Delhi, India
1993	Research Associate, Council of Scientific and Industrial Research (CSIR), New Delhi, India
1995	Science and Technology Agency Fellowship (STA Fellowship), Research and Development Corporation of Japan (JRDC), Japan through Japan International Science and Technology Exchange Center

1996 Pool Officer, Council of Scientific and Industrial Research (CSIR), New Delhi, India
2010 Innovation Excellence Award, Drug Discovery Division, Southern Research Institute, Birmingham, AL

C. Contribution to Science

1. During my Post-doc, I was trained as an organic/medicinal chemistry, firstly as a natural product chemist in artemisinin project that resulted in antimalarial drug Emal (Arteether), and then in asymmetric synthesis using chiral auxiliaries. During my first independent position as Research Scientist at SR, I was involved in developing disaccharide probes/inhibitors to study glycosyltransferases in cell wall of *Mycobacterium tuberculosis*. These work has produced several publications and presentations.
    1. A.K. Pathak*, V. Pathak and R.C. Reynolds*. Solution Phase Parallel Synthesis of Acyclic Nucleoside Libraries of Purine, Pyrimidine and Triazole Acetamides. ***ACS Comb. Sci.*** 16, 485−493 (2014). PMID: 24933643
    2. A.K. Pathak, V. Pathak, L.E. Seitz, W.J. Suling and R.C. Reynolds*. 6-Oxo and 6-thio purine analogs as antimycobacterial agents. ***Bioorg. Med. Chem.*** 21, 1685-1695 (2013). PMID: 23434367
    3. K.C. Reddy, N. Padmaja, V. Pathak and A.K. Pathak*. Concise synthesis of an arabinofuranose hexasaccharide present in the cell wall of *Mycobacterium tuberculosis.* ***Tetrahedron Lett.*** 53, 2461–2464 (2012).
    4. A.K. Pathak, V. Pathak, W. J. Suling, J. R. Riordan, S. S. Gurcha, G. S. Besra and R. C. Reynolds*. Synthesis of deoxygenated (α 1→5)-linked arabinofuranose disaccharides as substrates and inhibitors of arabinosyltransferases of *Mycobacterium tuberculosis*. ***Bioorg. Med. Chem.*** 17, 872-881 (2009). PMID: 19056279

2. I lead medicinal chemistry efforts in a company contract to design and synthesize Quillaic acid saponin analog GPI-0100 as vaccine adjuvant which is in Phase-III clinical trial for several anti-viral, antibacterial and anti-cancer vaccines. Later, independently developed analogs of Quillaic acid and Gypsogenin as immune agonists to be used in vaccines under two R21 grants funded through NIAID, NIH as PI (articles are being submitted). Work is still in progress to develop some hybrid adjuvant based on a hypothesis that synergy of two or more agonist ligands will activate different compartment of immune system with enhanced effects. During the development of this project new method to assemble oligosaccharides was also developed which is been used to assemble oligosaccharides of biological interests.
    1. A.K. Pathak, V. Pathak and R.D. May. Vaccine compositions for Marburg virus. US Patent Publication No. US20120136142.
    2. C.K. Yerneni, V. Pathak and A.K. Pathak*. Imidazolium cation supported solution-phase assembly of homo-linear α(1→6)-linked octamannoside – An efficient alternate approach for oligosaccharide synthesis. ***J. Org. Chem.*** 74, 6307–6310 (2009). PMID: 19624152
    3. D.J. Marciani*, R.G. Ptak, T.G. Voss, R.C. Reynolds, A.K. Pathak, T.L. Chamblin, D.R. Scholl and R.D. May. Degradation of *Quillaja saponaria* Molina saponins: Loss of the protective effects of a herpes simplex virus 1 sub-unit vaccine. ***Int. Immunopharmacol.*** 2, 1703-11 (2002). PMID: 12469944
    4. D.J. Marciani*, J.B. Press, R.C. Reynolds, A.K. Pathak, V. Pathak, L.E. Gundy, J.T. Farmer, M.S. Koratich and R.D. May. Development of semisynthetic triterpenoid saponin derivatives with immune stimulating activity. ***Vaccine***, 18, 3141 (2000). PMID: 10856794

3. I lead medicinal chemistry efforts in several projects in anti-infective drug discovery area funded in-house or through NIH. Inhibition of bacterial biofilm is an important target and my lab has developed some quinazoline based molecules which are being further developed as leads. My group is also involved in projects on anti-viral drug discovery and currently I lead medicinal chemistry core in Antiviral Drug Discovery and Development Center funded through NIAID, NIH. We are developing hits from

highthroughput screens against flavi, corona, alpha and influenza viruses. These hit molecules can be used as probes to study viral targets and will be further developed as lead molecules to treat the infections.

1. C.W. Evans, C. Atkins, A.K. Pathak, B.E. Gilbert, J.W. Noah*. Benzimidazole analogs inhibit respiratory syncytial virus G protein function. ***Antiviral Res.*** 121, 31-38 (2015). PMID: 26116756
2. L. Rasmussen, E. L. White, A. Pathak, J. C. Ayala, H. Wang, J.-H. Wu, J. A. Benitez and A. J. Silva*. A High Throughput Screening Assay for Inhibitors of Bacterial Motility Identifies a Novel inhibitor of the Na+-driven Flagellar Motor and Virulence Gene Expression in Vibrio cholera. *Antimicrob. Agents Chemother.* 55, 4134-4143 (2011). PMID: 21709090
3. L. Wen*, J. N Chmielowski, K. C. Bohn, J.-K. Huang, Y. N. Timsina, P. C. Chand and A. K. Pathak. Functional expression of *Francisella tularensis* FabH and FabI, potential antibacterial targets. ***Protein Expr. Purif.*** *65*, 83-91 (2009). PMID: 19095065
4. A.K. Pathak, V. Pathak, L.E. Seitz, W.J. Suling and R.C. Reynolds*. Antimycobacterial agents. 1. Thio analogs of purine. ***J. Med. Chem.*** 47, 273-276 (2004). PMID: 14695841

Complete List of Published Work in My Bibliography:

http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/44204623/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

Southern Research Institute sponsored Internal Research Projects

1U19AI109680-01 Prof. R.J. Whitely (PI) 03/01/2014 – 02/28/2019
NIH/NIAID
Antiviral Drug Discovery and Development Center
The herein proposed Center of Excellence for Translational Research (CETR), which will be named the Antiviral Drug Discovery and Development Center (AD3C) has, at its center, the theme to develop new small molecule therapeutics for emerging and re-emerging viral infections. Translational research will focus on the inhibition of viral replication, especially viral polymerase.
Role: Medicinal Chemistry Core PI/Senior Medicinal Chemist

Completed Research Supports

1R21AI101924-01 Ashish K. Pathak (MPI, Contact PI) 08/06/2012 – 07/31/2015
NIH/NIAID
Synthetic Gypsogenin Saponins as Synergistic Vaccine Adjuvants
The major goal of this project was to develop semi-synthetic Gypsogenin saponins as synergistic immune agonists and vaccine adjuvants.
Role: Principal Investigator in a Multi-PI grant (Other PI: Dr. Michael J. Fuller)

URC Grant Ashish K. Pathak (PI) 5/1/2008 – 04/30/2009
University Research Council (URC), Western Illinois University
Structurally Diverse Indole-2-carboxylic acid Analogs as Inhibitors of Fatty Acid Synthase (FAS) Enzymes in *F. tularensis*
The major goal of this project was to synthesize indole derivatives and evaluate their inhibitory activity against *F. tularensis* FabH and FabI
Role: Principal Investigator

1R21 AI059270-01 Ashish K. Pathak (PI) 5/01/2004 - 04/30/2008
NIH/NIAID
New adjuvant technologies for Marburg virus vaccine